Biofilm formation on surface modified silicone rubber voice prostheses

Everaert, Emmanuel Paul Jos Marie

Biofilm formation on surface modified silicone rubber voice prostheses

Emmanuel P.J.M. Everaert

Rijksuniversiteit Groningen

Biofilm formation on surface modified silicone rubber voice prostheses

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus Dr. F. van der Woude
in het openbaar te verdedigen op
woensdag 10 september 1997
des namiddags te 4.15 uur

door

Emmanuel Paul Jos Marie Everaert
geboren op 29 april 1966
te Namen, België

Groningen
1997

Promotores:	Prof.dr. J. Arends
	Prof.dr. H.F. Mahieu
Co-promotor:	Dr.Ir. H.J. Busscher
Referent:	Dr. H.C. van der Mei

ISBN 90-6523-087-4

Stellingen

behorende bij het proefschrift

Biofilm formation on surface modified silicone rubber voice prostheses

Emmanuel P.J.M. Everaert

1 De mate van oppervlakte-crosslinking van siliconenrubber kan worden bepaald door middel van randhoekmetingen.

Dit proefschrift

2 Het gebruik van de "split-button" is een elegante methode om *in vivo* de biofilm formatie van een behandelde stemprothese te beoordelen.

Dit proefschrift

3 Siliconenrubber oppervlakken kunnen permanent hydrofiel worden gemaakt.

Dit proefschrift

4 De hydrofobiciteit van siliconen rubber wordt sterk vergroot door chemiesorptie van perfluorotrichlorosilanen.

Dit proefschrift

5 Het chemisch behandelen van siliconenrubber met lange-keten perfluoro-alkylsiloxaan vergroot de mogelijkheid micro-organismen te verwijderen.

Dit proefschrift

6 Het toevoegen van antibiotica aan siliconenrubber van stemprotheses gaat ten koste van de mechanische eigenschappen.

7 Siliciumconcentraties oplopend tot 70 %, zoals berekend met behulp van XPS-diepte profiel metingen aan siliconen rubber door Silver et al., hebben geen chemische betekenis.

Silver et al. (1995), J. Biomed. Mat. Res. 29:535-548.

8 Het aanklagen van Dow Chemicals voor het mislukken van borstprothesen is medisch gezien een ramp.

P M Galleti (1996), J. Biomed. Mat. Res., 32:289-291.

9 Gelaryngectomeerde patiënten hebben ook stemrecht.

10 De levensduur van een stemprothese hangt mede af van de gebruiksfrequentie.

11 Zeggen dat vaak onder de zonnebank liggen om niet te verbranden in de zon, is onjuist.

12 Het feit dat de chemie en de chemische industrie een slecht imago hebben wijst erop dat het belang van kennisoverdracht en van het vermogen om de essentie van wetenschappelijk en industrieel onderzoek te kunnen weergeven wordt onderschat.

13 Een proefschrift wordt door collega's teveel gewaardeerd op het feit of ze wel of niet in het dankwoord worden genoemd.

14 Bij het voorschrijven van normen voor de aanwezigheid van schadelijke stoffen in voedingsmiddelen dient niet te worden uitgaan van wat hallbaar is voor de producent, maar van wat wenselijk is voor de consument.

15 Ter bestrijding van de zure regen zou het circuit van zandvoort omgebouwd moeten worden tot een baan voor marathon-skeelers en schaatsers, dit afhankelijk van het seizoen.

Groningen, 10 september 1997

Leescommissie: Prof.dr. F.W.J. Albers
Prof.dr. J. Feijen
Prof.dr. A. van Nieuw Amerongen

Paranimfen: Sabine Meijer
Angélique M. Reitsma

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Everaert, Emmanuel P.J.M.

Biofilm formation on surface modified silicone rubber voice prostheses / Emmanuel P.J.M. Everaert,
-[S.l.:s.n.].-
Foto's
Proefschrift Groningen. Met lit. opg. - Met samenvatting in het Nerderlands en in het Frans.
ISBN 90-6523-087-4
Trefw.: laryngectomie; siliconenrubber; oppervlakte modificatie; stemprothese

Printing of this thesis was financially supported by:

Medin Instruments and Supplies, Groningen, The Netherlands
Atos Medical AB, Hörby, Sweden
Dow Corning, Sophia Antipolis, France
Entermed B.V., Woerden, The Nethelands
INHEALTH Technologies, Carpinteria, CA, USA
Janssen-Cilag B.V., Tilburg, The Netherlands
Nerderlandse Vereniging voor Biomaterialen (NVB)
NuSil Silicone Technology, Anglet, France

Printed by Uitgeverij van Brug, Groningen
The cover was designed by Aly Pepping, Thesingue

TABLE OF CONTENTS

PREFACE

At the congress "Bioadhesion II" held at the Université Catholique de Louvain (UCL) in Louvain-la-Neuve, 23-27 May 1993, Belgium, I read the following announcement:

> **At:** *Laboratory for Materia Technica, University of Groningen, The Netherlands*
>
> **Job:** *To develop silicone modifications that make the materials less adhesive for microorganisms and less susceptible to biodeterioration, when used as a voice prosthesis.*
>
> **Contact:** *Henk Busscher (...),*
> *Starting Sept. 93 /4 yr contract, Only for Ph-D students*

There were two other subjects, but within ten seconds my choice had been made. Groningen? Never heard about it... Anyway, a Ph-D position was something I really desired. Encouraged by Nava Mozes: "*It's a good lab, don't hesitate ...*", I applied. My interview took place at the terrace of a cafe on a very warm sunny day. I ordered a heavy Belgian beer. Trying to make himself clear by what added up to a handsome pile of drawings, Henk explained to me what I was supposed to do. The next day he asked me to call him back one week later. I did. On June 17th 93, I visited his lab. On August 31th, I packed my car and left Belgium for Groningen. Only when I saw the road sign "Groningen 14 Km" on the highway, did I realize that something totally new was about to begin. Less than four years later, my Ph-D thesis would not have been achieved without the assistance of the following persons, each of whom I want to thank in his or her own language:

Alleerst, wil ik graag de gelegenheid nemen om Henk Busscher te bedanken voor zijn grote enthousiasme voor mijn onderwerp en zijn verstandige commentaar op de verschillende versies van al mijn artikelen. Henk, zonder jouw hulp zou het proefschrift nooit geworden zijn wat het nu is.

Henny van der Mei, ik tankje dy tige foar dyn grutte help by it praktyske wurk yn it lab, yn't bisûnder by it mikrobiële wurk dat nedich wie by de in-vivo eksperimenten en foar dyn krityske each by it goedkarren fan alle figueren. Troch dy stiet nou by elke figuer krekt itselde lettertype.

Hans Mahieu van KNO, VU zienkenhuis, Amsterdam, bedankt voor de uitstekende manier waarop je met ons hebt samengewerkt bij onze in-vivo experimenten. De vele goede gesprekken tot diep in de nacht die we samen in Sydney hebben gevoerd zal ik niet snel vergeten.

Joop Arends, uw bijdrage en belangstelling hebben ervoor gezorgd dat alle plannen zonder problemen verwezenlijkt konden worden.

De leescommissie, Prof. Albers, Prof. Feijen and Prof. van Nieuw Amerongen wil ik graag bedanken voor de snelle beoordeling van dit proefschrift.

Angélique Reitsma van KNO, VU zienkenhuis, Amsterdam, jouw grote betrokkenheid bij onze in-vivo experimenten heeft er belangrijk toe bijgedragen dat we deze succesvol af hebben kunnen sluiten. Hiervoor wil ik je hartelijk bedanken. Het was altijd een plezier om met jou samen te werken.

Bart Verkerke (BMTC, RUG, Groningen), jou wil ik graag bedanken voor de nuttige hulp die ik vaak van je gekregen heb. Martin de Vries en professor H.K. Schutte, bedankt voor het mogen gebruiken van de scanner op jullie afdeling.

Ron C. Chatelier and Prof H. J. Griesser (CSIRO, Clayton, Australia), I am grateful for the invitation to visit your lab in Melbourne. Ron, our cooperation has resulted in chapter 4 of this thesis and for this I would like to thank you once more.

Kees Rinzema, fjouwer jier haw ik genietsje kinnen fan de och sa gesellige sfear op ús keamer. It is my in ear om dy hjirfoar te bitankjen. Ek soe ik graach mei dy yn kontakt bliuwe. Kees, ik winskje dy in hiel soad súkses yn't libben.

Sabine Meijer, 't kleine schatje, jouw aanwezigheid heeft mijn laatste M.T.-jaar extra kleur gegeven. Bedankt voor alle interessante gesprekken die we in Bar Volonté hebben gevoerd.

Joop de Vries, dien vlaauwe Belg'nmoppen heb'k nait altied in gelieke moate wurdeerd, moar'k wil die e'emgoud dankzeggen veur alle XPS-metings en computerhulp. Betsy van de Belt, bedankt voor alle hulp bij het kweken en het overige in-vivo werk.

Freark Dijk (EM, RUG), tankewol foar alle SEM-foto's en alle preparaasjes fan de stimprotheses dy't dêrfoar nedich wienen.

Sabine Zels, Ich danke dich für die drei Jahren daß wir zusammen waren. Das war einmal aber ich vergesse es nicht. Auch danke ich deine Eltern für das Vertrauen und die Gemütlichkeit der ich bekommen habe. Zum Scluß mochte ich gerne alle Freunde aus Oldenburg und Augustfehn bedanken.

Al die medewerkers van Materia Technica, met name Teun, Kevin, Jan, Martine ..., die op één of andere manier hun betrokkenheid bij mijn persoonlijke wederwaardigheden hebben getoond wil ik graag bedanken voor hun bijdrage aan het veraangenamen van mijn verblijf in Groningen.

Verder bedank ik alle vriendinnen en vrienden, met name Evelyne en Thomas, Deanna, Carla, Jitske, Alain en Sylvianne, Rose-marie, Ahn, Ultimate Gronical Dizziness ..., die mijn Groningse jaren tot een onvergetelijke tijd hebben gemaakt.

Je remercie également mes parents pour toute l'aide qu'ils m'ont procurré avec ferveur durant ces quatre années que j'ai passées dans la belle ville de Groningue.

Emmanuel

Groningen, 4 juli 1997

Research reported in this thesis was carried out at the
University of Groningen, Laboratory for Materia Technica, Bloemsingel 10, 9712 KZ Groningen,
The Netherlands. Phone: +31 (0)50 3633140. Fax: + 31 (0)50 3633159.

This project was funded, in part, by a grant from the European Community, Eureka project EU72311.

GENERAL INTRODUCTION

LARYNGECTOMY AND PROSTHETIC VOICE RESTORATION

Introduction Laryngeal and pharyngeal cancers are by far the most frequently occurring cancers in the upper airway and digestive tract. Reported incidences in Europe vary between 11.4 per 100,000 inhabitants (Italy, Spain) to less than half that number in the Netherlands and Denmark depending upon etiological background (i.e. smoking and drinking). Between 5 to 20% of these cancers will require treatment with total laryngectomy leaning on tumor stage and differing treatment protocols (i.e. radiotherapy vs. surgery).

The most disabling consequence of laryngectomy is generally considered to be the loss of vocal functions. The anatomy before and after laryngectomy is shown in Fig. 1. First, the larynx, including the vocal folds, is removed. Subsequently, the lower respiratory tract is separated from the vocal tract and from the upper digestive tract. The laryngectomee breathes through a tracheostoma, and the direct connection of the vocal tract with the upper digestive tract remains unaltered. After laryngectomy the patient is not only deprived of the vibrating sound source (the vocal folds), but also the energy source for voice production (air stream from the lungs) is no longer connected to the vocal tract. The laryngectomee has to develop a new sound and energy source in order to acquire a substitute voice.

It is hardly surprising that ever since the first laryngectomies performed by Watson in 1866 (Alberti, 1975) and by Billroth in 1873 (Gussenbauer, 1874), methods have been sought to restore the voices of laryngectomees. For more than a hundred years, several methods of substitute voice production have been developed (Mahieu, 1988). The tracheo-pharyngeal or tracheo-esophageal shunt methods are presently the most widely used and most successful techniques of surgical voice restoration following laryngectomy. The energy source in these shunt (also so-called "voice prosthesis") methods is expiratory pulmonary air, just as in normal laryngeal voice production. When during expiration the tracheostoma is occluded, either by a finger or a valve mechanism, the air flows from the trachea through the shunt. Subsequently, the air enters the vocal tract where remaining mucosal and muscular structures at the esophageal entrance (pharyngo-esophageal segment) function as an alternative voice production system. Such a prosthesis effectively prevents stenosis of the shunt, as well as aspiration due to leakage of food or saliva from the upper digestive tract through the shunt. These prostheses only function as one-way valves, which allows the passage of air from the trachea into the esophagus; voice prosthesis does not produce sound.

There are the different types of voice prostheses mentioned, *non-indwelling* (removable) devices, which have to be removed regularly for cleaning purposes, such as the Algaba (Algaba, 1987), Blom-Singer (Singer and Blom, 1981) and the *indwelling* devices, which remain in the stand for a longer period of time, such as Blom-Singer, Eska Herrmann (Herrmann and Kley, 1981),

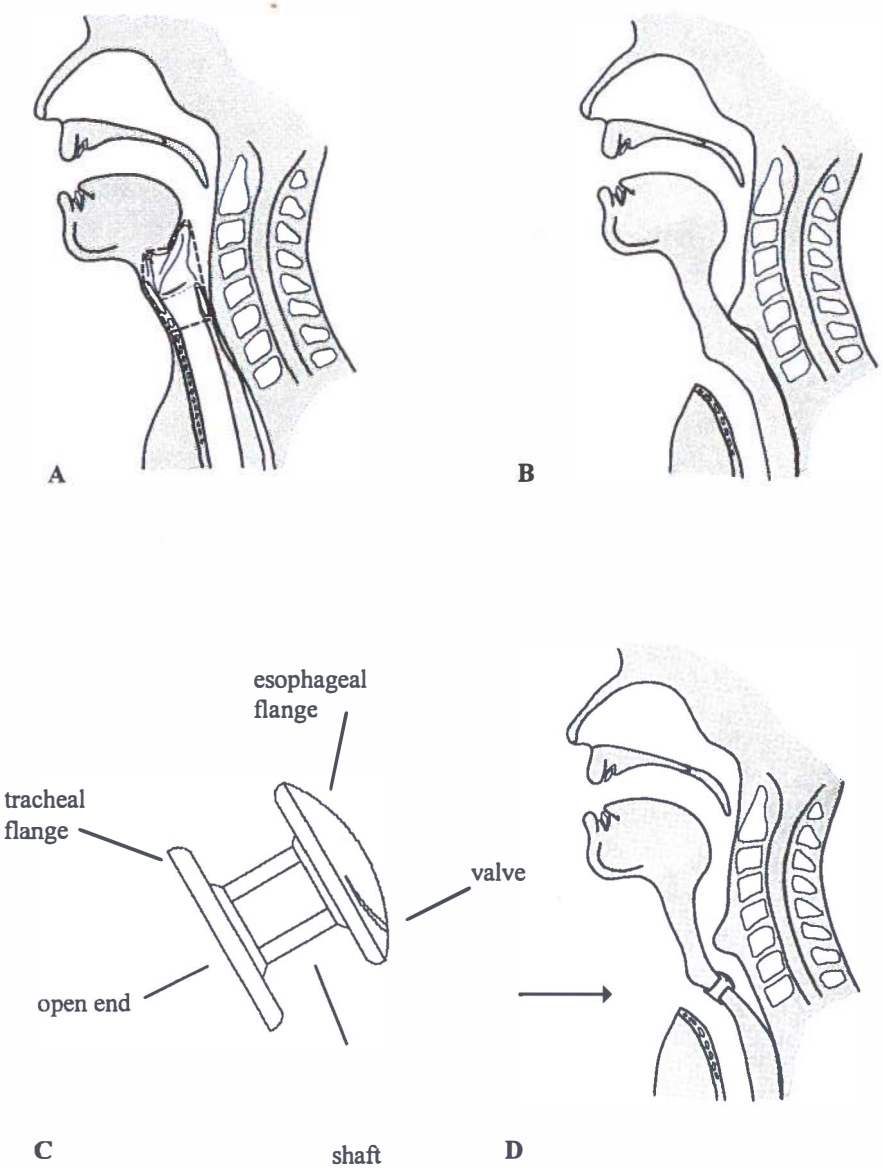


Figure 1. *Anatomy before and after total laryngectomy with resection lines marked (A). Note complete separation of the airway and digestive tract and the presence of a tracheostoma for respiration after laryngectomy (B) and the placement of i.e. a Groningen Button voice prosthesis (C) in the tracheo-esophageal shunt (D).*

Groningen button (Nijdam *et al.*, 1982; Mahieu *et al.*, 1987), Nijdam (Nijdam *et al.*, 1990), Provox™ (Hilgers and Schouwenburg, 1990; Hilgers and Balm, 1993), Provox®2, Staffieri (Staffieri, 1988), Traissac (Traissac *et al.*, 1987), Voice Master voice prostheses (developed by Schouwenburg). All these voice prostheses are inserted in the tracheo-esophageal shunt; the Groningen button, Nijdam and Provox voice prostheses are interchangeable. Fig. 2 shows an example of two different types of voice prostheses the non-indwelling and the indwelling. Finally, Fig. 3 shows a typical endoscopic view of an esophageal flange of a Groningen button valve prosthesis and a view of the tracheal flange visible through the tracheostoma.

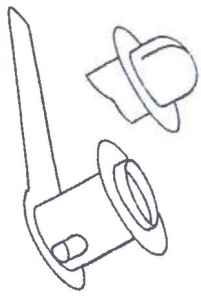
Indwelling voice prostheses are generally preferred by laryngectomees as many patients are inept due to lack of manual dexterity, fear or incomprehension to accurately remove and replace prostheses. Therefore, non-indwelling voice prostheses are especially allocated to motivated patients willing to be autonomous. Furthermore, removal and reinsertion of voice prostheses by the patient him/herself is associated with a higher complication rate (Mahieu, 1988). Often, the laryngologist and the patient have to opt for the best choice or compromise between non-indwelling or indwelling voice prostheses. In the United States, e.g. where health service is expensive and patients have to overcome large distances to reach a laryngologist, non-indwelling prostheses are more frequently used than in Western Europe.

Microbial colonization of voice prostheses

It has been well-documented that microorganisms can colonize surfaces of synthetic biomedical devices *in vivo*, resulting in disruption of prosthetic devices and sometimes in infection (Elliot, 1988; Gilsdorf *et al.*, 1989; Kristinsson, 1989), although such an infection is rarely seen in the use of voice prostheses.

Except for the Traissac voice prosthesis made of polyurethane and Eska Herrmann and the Voice Master combining metal and silicone rubber, all voice prostheses are mainly made of medical grade silicone rubber because of its excellent mechanical and molding properties. However, silicone rubber materials have the tendency to become quickly colonized by microorganisms (Neu *et al.*, 1993; Busscher *et al.*, 1996), most notably *Candida* species (Mahieu *et al.*, 1986; Palmer *et al.*, 1993; Neu *et al.* 1994; Natarajan *et al.*, 1994; Ell *et al.*, 1996; Everaert *et al.*, 1997) resulting in frequent replacement of indwelling prostheses, on average every four months (Van den Hoogen *et al.*, 1996). The microflora isolated from voice prostheses commonly consists of *Candida* species, *Streptococci* and *Staphylococci*, as summarized in Table I. Although *Candida* species are mainly held responsible for microbial overgrowth of prostheses, the role of bacteria has recently been emphasized again (Ell *et al.*, 1996). Ell and co-workers studied the microflora of 55 failed Groningen buttons. In case of valve

non-indwelling voice prostheses



The Blom-Singer duckbill voice prosthesis

The Blom-Singer low-pressure voice prosthesis

indwelling voice prostheses

Figure 2. *Different types of voice prostheses used in the tracheo-esophageal shunt of laryngectomized patients. (Top) non-indwelling Blom-Singer, (bottom-left) indwelling ultra-low resistance Groningen button and (bottom-right) Provox™ voice prostheses.*

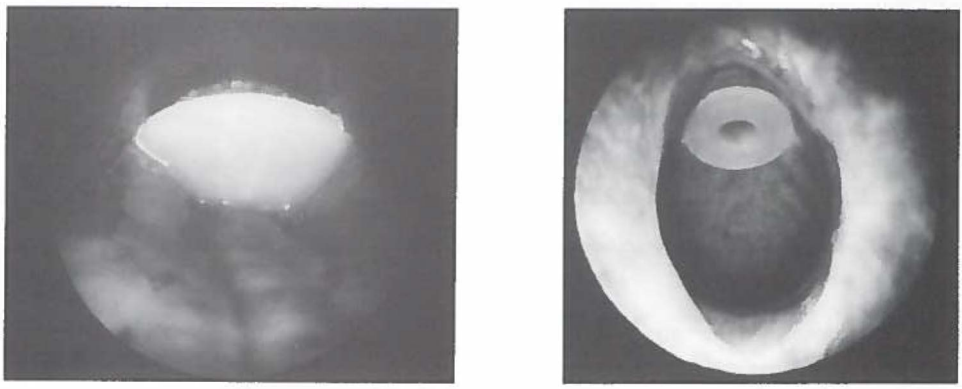


Figure 3. *Endoscopic view of the esophageal flange (left) and view of the tracheal flange visible through the tracheostoma (right) of a Groningen button voice prosthesis inserted in the tracheo-esophageal shunt.*

failure due to leakage (n=25) there was a positive correlation between biofouling in the lumen of the valve and the number of *Streptococci* cultured. In valves failing due to increased air flow resistance, *Enterococci* were particularly found on the esophageal surface.

The oropharyngeal cavity can be considered as the source of the *Candida* organisms. Oropharyngeal contents contaminated with yeasts are continuously swallowed. *Candida* organisms seem to possess a high affinity for silicone rubber, resulting in adherence and invasive growth into the button. Moreover, in almost all patients needing a device change, a dense microbial deposit (yellow-brownish) was found particularly affecting the esophageal flange (Mahieu *et al.*, 1986; Neu *et al.* 1994; chapters 5 and 6).

Prolonging the life-time of voice prostheses

Voice prostheses are replaced when, due to biofilm formation, laryngectomees complain about leakage of food and liquid or, though less often, increased air flow resistance of the valve (Izdebski *et al.*, 1987; Mahieu, 1988). In this context, anti-fouling improvement of the silicone rubber material is desirable, and various other pathways have been attempted to retard biofilm formation on indwelling voice prostheses (Mahieu *et al.*, 1986; Frosh *et al.*, 1996; Van Weissenbruch *et al.*, 1997). To achieve

selective decontamination of the oropharynx by daily administration of an antimycotic drug, has been tried. Mahieu and co-workers (1986) succeeded in reducing *Candida* colonization of voice prostheses by eliminating the oropharyngeal yeast source with an administration (10 mg four times daily) of

Table I. Bacterial and yeasts strains most frequently isolated from silicone rubber voice prostheses removed from laryngectomized patients.

identification and comments	prosthesis type	n*	reference
- *Candida* species - *Staphylococcus aureus*	Groningen button	10	Mahieu *et al.* (1986)
- *Candida albicans* - *S. aureus*	Blom-Singer	44	Palmer *et al.* (1993)
- *C. albicans*	Provox	3	Natarajan *et al.* (1994)
- *C. albicans, C. tropicalis* - unidentified cocci	Eska-Herrmann	7	Neu *et al.* (1994)
- *C. albicans, C. tropicalis, C. glabrata, C. krusei* - *Streptococcus mitis, S. salivarius, S. equisimilis* - *S. aureus, S. epidermidis*	Groningen button	26	Neu *et al.* (1994)
- *Candida* species - *S. aureus* - *Pseudomonas aeruginosa* - *streptococci* - *Escherichia coli*	Cannulas of T-tubes		Brown and Montgomery (1996)
- *Candida* species - *staphylococci* - *streptococci* - *enterococci*	Groningen button	55	Ell et al. (1996)
- *C. albicans, C. glabrata, C. krusei* - *S. mitis, S. salivarius* - *S. aureus, S. epidermidis*	Groningen button	7	Everaert *et al.* (1997)
- *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. guillermondi* - *S. aureus*	Provox	55	Van Weissenbruch *et al.* (1997)

*n = number of buttons used

amphotericin B lozenges. However, it was also demonstrated that not all patients regularly used the medication as prescribed. It was concluded that the daily administration of antimycotic medication is not the ideal solution for preventing silicone rubber voice prosthesis dysfunction. Other researchers attempted to retard biofilm formation on indwelling voice prostheses with varying degrees of success by daily intake of 2 liters of Turkish yoghurt or Kephir containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, or by the use of a buccal bioadhesive slow-release tablet containing antimycotic agents, such as miconazole-nitrate (Van Weissenbruch *et al.*, 1996). However, long term use of antimycotics may induce the development of resistant strains with all associated risks.

Frequent prosthesis removal (i.e. use of a non-indwelling voice prosthesis) and cleaning with antimycotic solutions or detergents and water has been recommended (in the past) to prevent the valve deterioration (Blom and Singer, 1986; Izdebski *et al.*, 1987; Modica, 1987). However, Mahieu (1988) and co-workers doubted this remedy, because frequent removal of voice prostheses by the patient is known to be associated with a higher complication rate. Moreover, Herrmann *et al.* (1986) concluded that once adhesion and invasion of *Candida* species into the silicone material had taken place, it was impossible to remove those yeasts without damaging the prosthesis.

MICROBIAL BIOFILMS

Introduction

Microorganisms not only colonize medical implants but even grow on metal surfaces such as copper which was formally considered toxic to all microorganisms (Jolley *et al.*, 1988). Apparently, biofilm formation on surfaces is a natural phenomenon and will arise wherever suitable conditions of moisture, temperature and nutrition exist. There is no simple definition of biofilms since they can vary dramatically in geometry and composition. Nevertheless, Marsh and Martin (1992) defined oral biofilms as a variety of microbial strains and species, embedded in a matrix of salivary and microbial components. Alternatively, Wimpenny (1994) defined a biofilm as "a predominantly two-dimensional microbial community which forms at a solid/liquid interface and which may become spatially heterogeneous by virtue of physico-chemical gradients that develop within it". Finally, Costerton and co-authors (1995) defined a biofilm as "matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces".

It is well accepted that all biofilms originate from the same sequence of events (Van Loosdrecht *et al.*, 1990; Bos, 1996; Busscher *et al.*, 1996) as illustrated in Fig. 4. When microorganisms and (bio)materials surfaces are in an aqueous environment, in which organic matter is present (e.g. sea water, milk, saliva, urine, or blood), (bio)material surfaces are first covered by a so-called "conditioning film". Proteins and other organic molecules will adsorb to the biomaterial surface prior

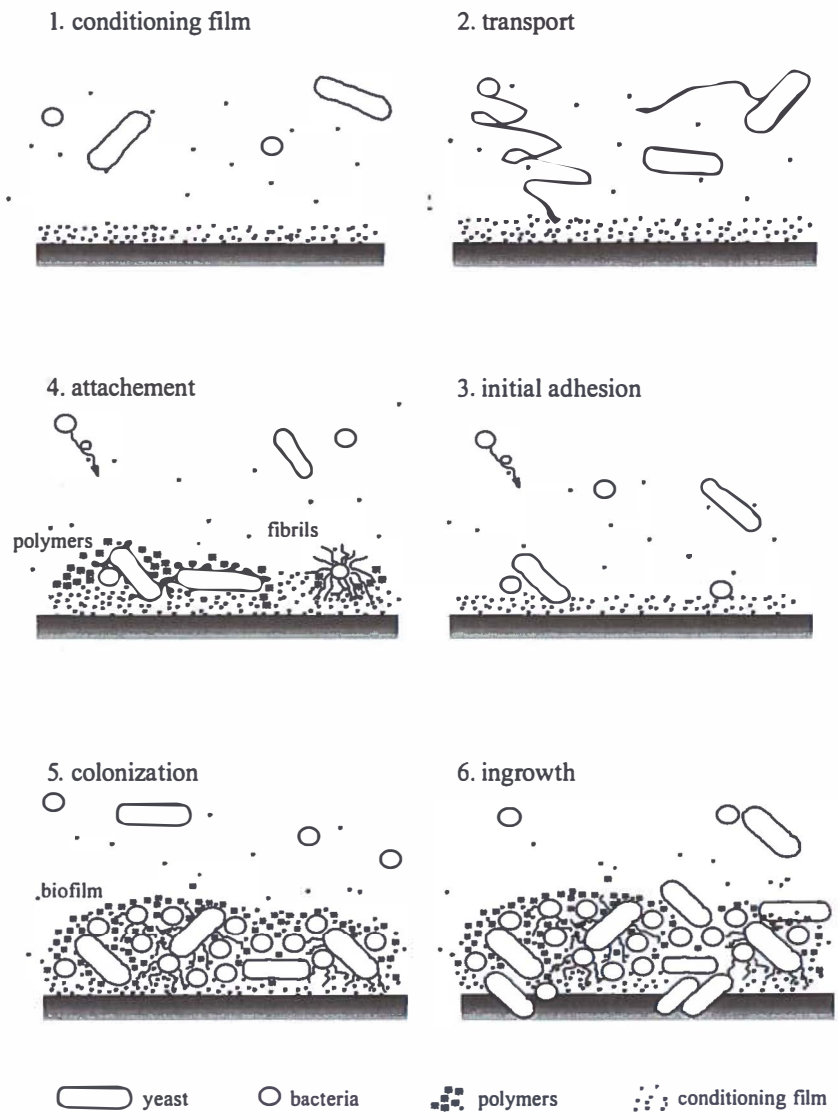


Figure 4. *Sequential steps in the formation of a biofilm on biomaterials used for voice prostheses and subsequent biodegradation by ingrowing yeasts.*

to arrival of the first microorganisms because proteins diffuse much faster than microorganisms. As a second step, the microbial transport can occur by different mechanisms such as Brownian motion, setting under gravity, diffusion, convection, or the intrinsic mobility of the microorganisms. Also,planktonic (in medium) microorganisms may be transported to each other and microbial coaggregates can be formed. Subsequently, microbial adhesion may occur which is often initially reversible. Later on, microbial anchoring occurs through exopolymer production, i.e. polysaccharides (Sutherland, 1977; Neu and Marshall, 1990) yielding firm, irreversible, adhesion of microorganisms. A few adhering sessile (on surfaces) microorganisms can stimulate further adhesion of other planktonic microorganisms, through strong attractive interactions between sessile and planktonic microorganisms (Busscher & Weerkamp, 1987). Finally, as shown in Fig. 4, adhering microorganisms start growing, possibly followed by ingrowth of selected microorganisms in the colonized material.

The majority of bacteria in natural environments are found attached to surfaces and not suspended in the aqueous phase as planktonic bacteria (Lappin-Scott & Costerdon, 1989). From many direct observations in medical, dental, industrial and agricultural areas of microbial ecology we have to conclude that microbial growth on surfaces dominates over planktonic growth in virtually every oligotrophic environment.

Physico-chemical mechanisms of initial adhesion

Busscher and Weerkamp (1987) described a three point hypothesis of bacterial adhesion mechanisms related to the distance of bacteria from a surface (Fig. 5). Firstly, at a distance of >50 nm from the surface Van der Waals forces are operatives. Secondly, both Van der Waals forces and electrostatic interactions occur together between 10 to 20 nm from the surface: this is associated with a change from reversible to effectively irreversible adhesion. Thirdly, at less than 1.5 nm from the surface Van der Waals forces, electrostatic interactions and specific interactions occur between bacteria and surface of the substrate, producing irreversible binding. The specific interactions may include the production of adhesive materials, such as exopolysaccharides.

In a thermodynamic approach in which electrostatic interactions are neglected adhesion can be predicted from an interfacial free energy balance. So far, several approaches are used to estimate the interfacial free energy changes involved in the adhesion of microorganisms (Absolom *et al.*, 1979; Busscher *et al.*, 1984; 1990) to solid substrata. These approaches are either based on the equation of state (Absolom *et al.*, 1979) or on the concept of dispersion and polar components sometimes interpreted by Van Oss *et al.* (1987) as Lifshitz-Van der Waals and acid-base components, respectively (Van Oss *et al.*, 1986; 1987).

The thermodynamic theory considers the surface free energies of the substratum, the microbial

cell surface and the suspending medium. Subsequently, these three surface free energies can be used to calculate the interfacial free energies between the interacting surfaces (Fig. 6). Accordingly, this comparison is expressed in the so-called free energy of adhesion

$$\Delta G_{adh} = \gamma_{sm} - \gamma_{sl} - \gamma_{ml} \qquad (1)$$

in which γ_{sm} , γ_{sl} , and γ_{ml} , are the solid-microorganism, solid-liquid, and microorganism-liquid interfacial free energies, respectively. Adhesion will be favourable if ΔG_{adh} is negative, since systems tend to minimize their free energy. Accordingly, an ideal anti-fouling surface should have a $\Delta G_{adh} > 0$. Consequently, influencing γ_{sm} and γ_{sl} by means of surface modification, could be a possible pathway to improve the anti-fouling properties of silicone rubber. Applying the thermodynamic theory, some investigators, have demonstrated that variations in thermodynamic parameters can result in corresponding and sometimes predictable modifications in microbial adhesion (Absolom, 1988; Busscher *et al.*, 1986; Fujioka-Hiray *et al.*, 1987).

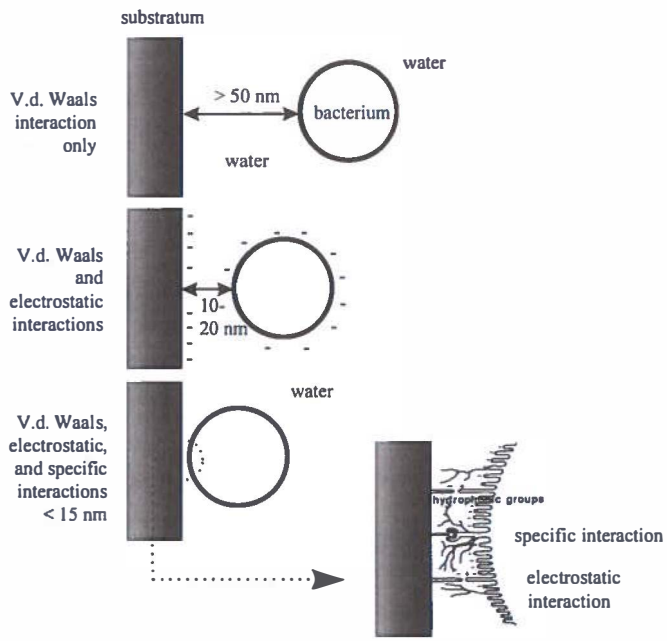


Figure 5. *Three point hypothesis of bacterial adhesion mechanisms related to the distance of the bacterium from the substrate. (Busscher & Weerkamp, 1987).*

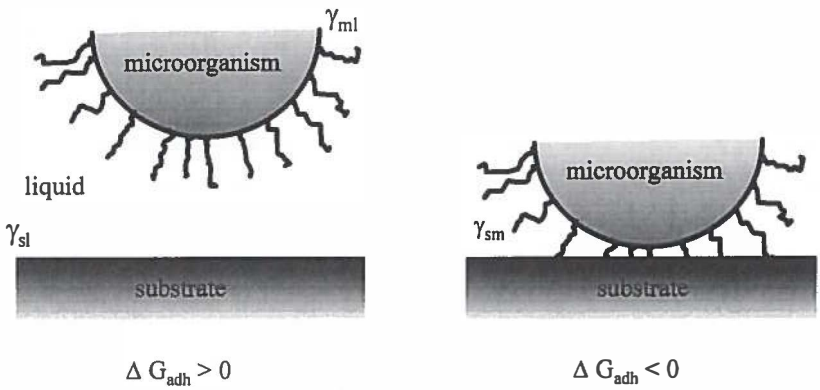


Figure 6. *Schematic presentation of the interfacial free energies γ_{ij} involved in the adhesion of a microorganism to a solid substrate surface from a liquid suspension. Indicated are the solid-microorganism (γ_{sm}), the solid-liquid (γ_{sl}), and the microorganism-liquid (γ_{ml}) interfacial free energies.*

An alternative physico-chemical approach towards microbial adhesion is based on the so-called DLVO (Derjaguin, Landau, Verwey, Overbeek) theory. This includes Lifshitz-Van der Waals and electrostatic interactions and their decay with separation distance. However, the DLVO theory originally describes the kinetics of the adhesion process rather than the equilibrium situation (Rutter & Vincent, 1980). The interaction energy between a spherical particle and a flat solid substratum can be described as

$$G_{(d)}^{TOT} = G_{(d)}^{LW} + G_{(d)}^{EL} \tag{2}$$

with $G_{(d)}^{TOT}$, $G_{(d)}^{LW}$, and $G_{(d)}^{EL}$ denote the total, the Lifshitz-Van der Waals and the electrostatic interaction energy at a distance (d), respectively, in which

$$G_{(d)}^{LW} = -\frac{A}{6}\left\{\frac{2a\ (h+a)}{h(h+2a)} - \ln\left[\frac{h+2a}{h}\right]\right\} \tag{3}$$

$$G_{(d)}^{EL} = \pi\varepsilon\ a(\zeta_s^2+\zeta_m^2)\left\{\frac{2\zeta_s\ \zeta_m}{\zeta_s^2+\zeta_m^2}\ln\left[\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right] + \ln[1-\exp(-2\kappa h)]\right\} \tag{4}$$

A denotes the Hamaker constant, a the bacterial radius, h the interaction distance, ε the permittivity of the medium, ζ_s and ζ_m the zeta potentials of the substratum and microorganism, respectively, and

κ the reciprocal Debye-Hückel length. According to the DLVO approach, anti-fouling properties of a material not only include Lifshitz-Van der Waals ("hydrophobicity") but also electrostatic interactions.

In summary, the thermodynamic and DLVO approaches showed that initial microbial adhesion is governed by surface properties of the material used. Therefore, surface modifications could be a pathway to improve anti-fouling properties of a substrate.

MODIFICATIONS OF SILICONE RUBBER

Chemistry of silicone rubber

Silicon is the second most abundant element on earth and is always found as oxide in the form of quartz and silicates. Yet, no natural compounds with carbon-silicon bonds exist. Amorphous silicon was first isolated as an element in 1824 by Berzelius. In 1863, Friedel and Crafts synthesized the first carbon-silicon bonds; Kipping (1924) investigated the synthesis of silanes and silicones. In the 1930's a commercial significance of silicones started to arise. Nowadays, two major applications of silicon in production are hyperpure elemental silicon for semiconductors applications and the organosilicone products as fluids, resins and elastomers. The medically oriented use of these new materials came into sight in 1959 with the establishment of the Dow Corning Center for Aid to Medical Research. Improved versions of silicone rubber designed for medical use were developed to answer the wide variety of requests with the result that they are now one of the most widely used implantable synthetics. Recently, problems caused by some silicone implants such as women's breast prostheses, constrained Dow Corning to limit the production of Medical Grade silicone elastomers. Nowadays, NuSil Silicone Technology (Carpinteria, CA, USA) produces and trades the Medical and Implant Grade Silicone products and warrants identical products specificities. Note that "Implant" grade silicone rubber possess identical physico-chemical characteristics as compared to the medical grade. At present, medical-device manufacturers have to use "Implant" grade silicone rubber when devices are in contact with blood or implanted for a period longer than 3 weeks. Differences between implant and medical grade silicone rubber, are probably more to be found in the sales price (± Dfl 1600/kg), which is approximately 8 times higher for the implant grade due to the "bellowing" insurances.

The term "medical grade" is applied to those silicones that fulfil three requirements:

- a long history of successful implantation in animals and humans,
- manufactured under pharmaceutically clean conditions,
- quality controlled for medical application.

The silicone rubbers used for medical purposes (implant as well as medical grade) contain also fillers and cross-linking agents, but do not contain the wide variety of additives used in organic rubber

compounding such as colour pigments, plasticizers, antioxidizers and heat stabilizers. The fillers used are very pure, finely divided silica with a particle size of about 30 nm; without this filler the silicone rubber would have insufficient strength.

Silicones are based on the siloxane Si-O-Si backbone. They are noted for their uniform mechanical properties from very cold to very high temperatures, resistance to aging, low surface energies, hydrophobicity, good electrical insulating properties, chemical inertness.

The first step in the production of a silicone is the reduction of silica (quartz sand) to elemental silicon at high temperature (1700 °C):

$$SiO_2 + C \rightarrow Si + CO_2$$

The silicon is subsequently reacted with methyl chloride. Under proper conditions this results in dimethyl dichlorosilane:

$$Si + 2\ CH_3Cl \rightarrow (CH_3)_2SiCl_2$$

The dimethyl dichlorosilane can react with two molecules of water and form a diol:

$$(CH_3)_2SiCl_2 + 2\ H_2O \rightarrow (CH_3)_2Si(OH)_2 + 2\ HCl$$

The diol is extremely unstable and immediately condenses with a neighbour molecule to form water and a silicone polymer (polysiloxane), commonly known as polydimethylsiloxane (PDMS):

$$n\ (CH_3)_2Si(OH)_2 \rightarrow HO\text{-}[Si(CH_3)_2\text{-}O]_n\text{-}OH + n\ H_2O$$

The polymer chains are terminated by OH groups. However, when hexamethyl disiloxane

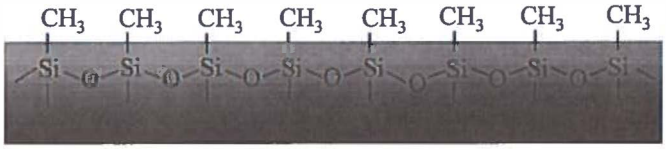
$$(CH_3)_3\text{-}Si\text{-}O\text{-}Si\text{-}(CH_3)_3$$

is added, the polymer chains are end-blocked by CH_3 groups so that predetermined average molecular weights can be obtained.

The "vulcanization" (cross-linking) of this polymer is simply the tying together of the hitherto separate siloxane polymer chains to yield a silicone rubber compound. Medical-grade silicone rubbers can be divided into two categories: the heat vulcanizing types (i.e. used for voice prostheses) and the room temperature vulcanizing types commonly called RTV's. The latter can be further divided into

those which vulcanize after addition of a catalyst and those which vulcanize by reaction with water absorbed from the air. The material used for voice prostheses production is made up of a two part enhanced tear resistant (ETR) silicone elastomers that consist of dimethyl and methylvinyl siloxane copolymers and reinforcing silica. Mixing the two components in the presence of a platinum catalyst (~1ppm) and increasing temperature (200 °C) initiates reaction of vinyl groups with siloxane polymer chains that results in the formation of cross-linked silicone. This copolymer is used because the methylvinyl portion makes for a more efficient cross-linking, yielding a rubber with good mechanical properties. Moreover, platinum catalyzed systems have the advantage of being solvent free and containing only trace amounts of catalyst.

In air, the top surface of untreated silicone rubber probably resembles of a layer of oriented, close-packed, methyl groups (Zisman, 1964):



The very high flexibility of the siloxane backbone allows the exposure, in air, of the methyl groups to their best effect leading to a relatively high value of the advancing water contact angle (115°± 3, Everaert *et al.*, 1995).

Modifications on biomaterials surfaces

Plasma treatment

Over the years, several methods have been developed to modify polymer surfaces for improved adhesion, wettability, printability and biocompatibility. Attempts include mechanical treatments, wet-chemical treatments, exposure to flames, corona discharges, and glow discharge plasmas. A basic objective of any such treatment is to remove loosely bonded surface contaminations and to provide intimate contact between the two interacting materials (reactant and substrate) on a molecular scale. Especially polymeric surfaces can be efficiently modified by a glow-discharge plasma treatment, in which a non-polymer forming plasma (i.e. plasma of argon, oxygen or nitrogen) is used. Briefly, a plasma may be defined as a partially ionized gas, with equal number densities of positive and negative charge carriers, in which the charged particles are "free" and possess collective behaviour. Plasma treatment deals with overall effects of very complex reactions and the processes are highly system dependent. Plasma treatments essentially modify composition and structure of a few molecular layers

at or near the surface of the material without affecting the bulk properties.

In a low-pressure (≤ 1 Torr), high-frequency (i.e. 13.56 MHz) discharge, the heavy particles (gas molecules and ions) are essentially at ambient temperature (kinetic energy ≈ 0.025 eV), while the electrons have enough kinetic energy (several eV) to break covalent bonds, and even to cause further ionization. The chemically reactive species thus created, can take part in homogeneous (gas-phase) or heterogeneous reactions with a solid surface in contact with the plasma (see Fig. 7). In the plasma treatment of polymers, energetic particles and photons generated in the plasma interact strongly with the polymer surface usually via free radical chemistry (Boenig, 1988; d'Agostino, 1990; Liston *et al.*, 1993). Since this type of plasma chemistry takes place at near-ambient temperature, it is well suited for processing thermally sensitive materials such as semiconductors and polymers (Hollahan & Bell, 1974; Boenig, 1988).

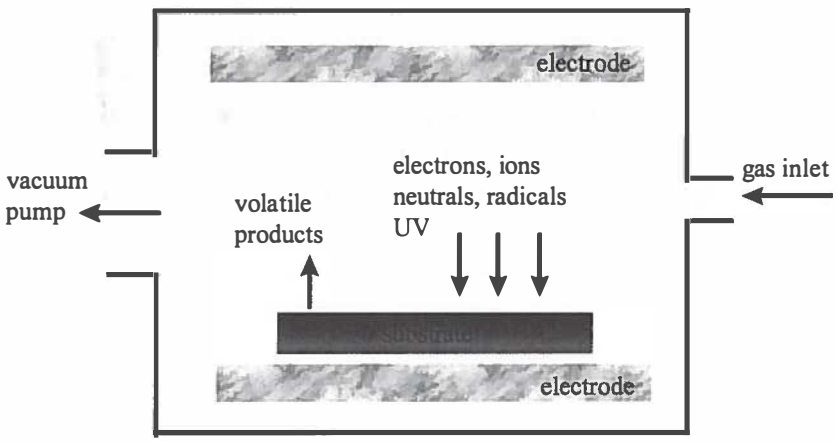


Figure 7. *Schematic presentation of the interaction of a substrate surface with a plasma.*

Plasma treatment has four major effects on surfaces. Each is always present to some degree, but one may be favoured over the others, depending on the substrate and the gas chemistry, the reactor design, and the operating parameters. The four major effects are:

(i) *surface cleaning* i.e.: removal of organic contamination from the surface;

(ii) *etching, or ablation*, of material from the surface, which can remove a weak boundary layer and increase the surface area;

(iii) *crosslinking or branching* of near-surface molecules, which can cohesively strengthen the surface layer; and

(iv) *modification of surface-chemical* structure, which can occur during plasma treatment itself, and upon re-exposure of the treated part to air, at which time residual free radicals can react with atmospheric oxygen or water vapour and creates new chemical functionalities.

Cleaning is one of the major reasons for improved bonding to plasma-treated surfaces. Most other cleaning procedures such as liquid rinse, leave a layer of organic contamination that may interfere with adhesion processes. However, any surfaces cleaned by plasma, will rapidly reacquire a layer of contamination when exposed to ambient air.

Etching is distinguished from cleaning only by the amount of removed material. Since amorphous polymers are removed many times faster than either its crystalline counterpart or inorganic filler material (i.e. SiO_2 filler for silicone rubber), a surface topography can be created, with the amorphous zones appearing as valleys. For example, plasma treatment of fluoropolymers (Kasemura *et al.*, 1990; Morra *et al.*, 1990) or polyethylene terephthalate (PET) (Hsieh *et al.*, 1989) for short times improves their wettability without modifying their surface texture; overtreatment gives a very porous surface. CASING (Crosslinking via Activated Species of Inert Gases) was one of the earliest-recognized plasma treatment effects on polymer surfaces (Hansen & Schonhorn, 1966). As suggested by the acronym, CASING occurs on polymer surfaces exposed to noble gas plasmas (i.e. argon or helium). They are effective at creating free radicals by breaking C-C or C-H bonds. If the polymer chain is flexible (like siloxane), or the radical can migrate along the polymer chain, this can give rise to recombination, unsaturation, branching, or crosslinking (Yasuda, 1985). Since all these processes can affect surface characteristics, it is crucial that one be able to characterize a given plasma treatment in terms of resulting changes in surface chemical composition, structure, biocompatibility, and physical or functional properties (Ratner, 1993; Liston *et al.*, 1993).

Molecules in plasma-modified polymers surfaces are often far from an equilibrium state and freshly modified polymer surfaces are unstable over time. Therefore, to minimize the free energy, the hydrophilicity created by a plasma is generally lost within hours to days (Garbassi *et al.*, 1989; Liston *et al.*, 1993; Owen and Smith, 1994; Chatelier *et al.*, 1995; Everaert *et al.*, 1995, 1996). This so-called "hydrophobic recovery" is caused, amongst others, by the mobility and reorientation of polymer chains in the treated surface layer. Therefore, storage conditions such as temperature and the hydrophobicity / hydrophilicity of the storage medium (i.e. in air or in water) will affect the kinetics and the final degree of the hydrophobic recovery of a plasma-treated polymer surface (Everaert *et al.*, 1996).

Other treatments on silicone rubber surfaces

Silicone polymers exhibit good biocompatibility and have been exploited for a variety of biomedical products (Arkles and Redinger, 1983). However, their success is often limited due to microbial fouling.

Therefore, several attempts to improve antifouling properties of silicone rubber devices have been made. Farber and Wolff (1993) demonstrated that silicone rubber catheters coated with salicylic acid reduced, in broth medium as well as in synthetic urine, bacterial adherence. Schierholz and co-workers (1994), incorporated an antibiotic, rifampicin, into the silicone. The last mentioned authors, reported that only the liberation of high antibiotic doses over a period of weeks could prevent the bacterial colonization of the silicone rubber devices. Boswald and co-workers (1995), concluded that catheters coated with silver significantly reduced, *in vitro*, bacterial adherence and that no cytotoxic or thrombogenic side effects was shown. Finally, tiny air nuclei found in the surface roughness of silicone rubber were removed by washing the material for 24h in EtOH at room temperature (Kalman *et al.*, 1991). The denucleated silicone rubber caused reduced platelet aggregation as compared to the control.

AIMS OF THIS THESIS

The aims of this thesis are:

- firstly, to characterize the physico-chemical properties of surface modified medical grade silicone rubber, in order to find a pathway to reduce the hydrophobic recovery occurring on freshly plasma-treated surfaces.
- secondly, to assess the *in vitro* effectiveness upon reducing microbial adhesion to the selected silicone rubber surface treatments based on the use of a parallel plate flow chamber system.
- thirdly, to develop a method to test the *in vivo* performance of surface treated silicone rubber voice prostheses with regard to biofilm formation.

REFERENCES

Absolom DR, Neumann AW, Zingg W, Van Oss CJ (1979), Thermodynamic studies of cellular adhesion. *Trans. Am. Soc. Artif. Intern. Org.* **25**: 152-156.

Absolom DR (1988), The role of bacterial hydrophobicity in infection, bacterial adhesion and phagocytic ingestion. *Can. J. Microbiol.* **34**, 287-299.

d'Agostino R (1990), In: Plasma deposition, treatment and etching of polymers. d'Agostino R (Ed). Academic Press, Boston.

Alberti PW (1975), The evolution of laryngology and laryngectomy in the mid 19th century. *Laryngoscope.* **85**:288-298.

Algaba J (1987), Récupération de la voix pour les patients laryngectomisés. *Revue de laryngologie.* **108**: 139-142.

Bergstrom K, Holmberg K, Safranj A, Hoffman AS, Edgell MJ, Kozlowski A, Hovanes BA, Harris JM (1992), Reduction of fibrinogen adsorption on PEG-coated polystyrene surfaces. *J. Biomed. Mat. Res.* **26**:779-790.

Blom ED, Singer MI (1986), Disinfection of silicone voice prostheses (letters to the editor). *Arch. Otolaryngol. Head Neck Surg.* **112**:1303.

Boenig HV (1988), In: Fundamentals of plasma chemistry and technology. Boenig (Ed). Technomic Publishing, Lancaster, PA.

Bos R (1996), Co-adhesion of oral microbial pairs a physico-chemical approach. Ph-D Thesis, University of Groningen, Groningen, The Netherlands. pp 196.

Boswald M, Girisch M, Greil J, Spies T, Stehr K, Krall T, Guggenbichler JP (1995), Antimicrobial activity and biocompatibility of polyurethane and silicone catheters containing low concentrations of silver: A new perspective in prevention of polymer-associated foreign-body-infections. *Zentrablatt fuer Bakteriologie* **283**:187-200.

Brown MT, Montgomery WW (1996), Microbiology of tracheal granulation tissue associated with silicone airway prostheses. *Ann. Otol. Rhinol. Laryngol.* **105**:624-627.

Busscher HJ, Weerkamp AH, van der Mei HC, van Pelt AWJ, de Jong HP, Arends (1984), Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. *Appl. Environ. Microbiol.* **48**:980-983.

Busscher HJ, Uyen MHWJC, van Pelt AWJ, Weerkamp AH, Arends (1986), Kinetic of adhesion of the oral bacterium *Streptococcus sanguis* CH3 to polymers with different surface free energies. *Appl. Environ. Microbiol.* **31**:910-914.

Busscher HJ, Weerkamp AH (1987), Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiol Rev.* **46**:165-173.

Busscher HJ, Sjollema J, van der Mei HC (1990), Relative importance of surface free energy as a measure of hydrophobicity in bacterial adhesion to solid surfaces. In: Microbial cell surface hydrophobicity. Doyle RJ, Rosenberg M (Eds). American Society for Microbiology, Washington DC. pp 335-359.

Busscher HJ, Geertsema-Doombusch GI, Everaert EPJM, Verkerke GJ, van de Belt-Gritter B, Kalicharan R, van der Mei HC (1996), Biofilm formation and silicone rubber surface modification in the development of a total artificial larynx. In: Surgery and Prosthetic voice restoration after total and subtotal laryngectomy. Algaba J (Ed), Elsevier Science, Amsterdam. pp 47-52.

Chang FY, Shen M, Bell AT (1973), Effects of electric discharge surface treatment on the diffusion characteristics of polymer. *J. Appl. Polym. Sci.* **17**:2915-2917.

Chatelier RC, Xie X, Gengenbach TR, Griesser HJ (1995), Effects of plasma modification conditions on surface restructuring. *Langmuir* **11**:2585-2591.

Chole RA, Brummett RE, Tinling SP (1995), Safety of silver oxide-impregnated silastic tympanostomy tubes. *Am. J. Otol.* **16**:722-724.

Colter KD, Bell AT, Shen M (1977), Reduction of progesterone release rate through silicone membranes by plasma polymerization. *Biomat. Med. Dev. Art. Org.* **5**:13-24.

Costerton JW, Ceng KJ, Geesey GG, Ladd Tl, Nickel JC, Dasgupta M, Marrie TJ (1987), Bacterial biofilms in nature and disease. *Annu. Rev. Microbiol.* **41**:435-464.

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995), Microbial biofilms. *Annu. Rev. Microbiol.* **49**:711-745.

Elimelech M (1990), Indirect evidence for hydratation forces in the deposition of polystyrene latex colloids on glass surfaces. *J. Chem. Soc. Faraday Trans.* **86**:1623-1624.

Elliott TSJ (1988), Plastic Devices: New fields for Old Microbes. *Lancet* **2**/9:30-31.

Ell SR, Davies CM, Clegg RT, Parker AJ (1996), Do bacteria play a role in silastic speaking valve failure?. In: Surgery and Prosthetic voice restoration after total and subtotal laryngectomy. Algaba J (Ed), Elsevier Science, Amsterdam. pp 363-365.

Everaert EPJM, van der Mei HC, de Vries J, Busscher HJ (1995), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 1. Storage in air. *J. Adhesion Sci. Technol.* 9:1263-1278.

Everaert EPJM, van der Mei HC, Busscher HJ (1996), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 2. A comparison of the hydrophobic recovery in air, water, or liquid nitrogen. *J. Adhesion Sci. Technol.* **10**:351-359.

Everaert EPJM, Mahieu HF, Wong Chung RP, Verkerke GJ, van der Mei HC, Busscher HJ (1997), A new method for in vivo evaluation of biofilms on surface-modified silicone rubber voice prostheses. *Eur. Arch. Otorhinolaryngol.* **254**:261-263.

Farber BF, Wolff AG (1993), Salicylic acid prevents the adherence of bacteria and yeast to silastic catheters. *J. Biomed. Mat. Res.* **27**:599-602.

Frosh AC, Cheesman AD, Perry A, McEwan J, Djazaeri B (1996), A mathematical model for cost-analysis in the use of amphotericin B with Blom-Singer prostheses. In: Surgery and Prosthetic voice restoration after total and subtotal laryngectomy. Algaba J (Ed), Elsevier Science, Amsterdam. pp 371-376.

Fujioka-Hiray Y, Akagawa Y, Minagi S, Tsuru H, Miyake Y, Suginoka H (1987), Adherence of *Streptococcus mutans* to implant materials. *J. Biomed. Mat. Res.* **21**:913-920.

Garbassi F, Morra M, Occhiello E, Barino L, Scordamaglia R (1989), Dynamics of macromolecules: a challenge for surface analysis. *Surf. Inter. Analysis* **14**:585-589.

Gibbons RJ (1980), Adhesion of bacteria to the surfaces of mouth. In: Microbial adhesion to surfaces. Berkeley RCW, Lynch JM, Melling J, Rutter PR, Vincent B (Eds). Ellis Horwood, Chichester. pp 351-378.

Gilbert P, Collier PJ, Brown MRW (1990), Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. *Antimicrobial agent and Chemotherapy* **34**:1865-1868.

Gilsdorf JR, Wilson K, Beals TF (1989), Bacterial colonization of intravenous catheter materials in vitro and in vivo. *Surgery.* **106**:37-44.

Gussenbauer C (1874), Ueber die erste durch Th. Billroth am Menschen ausgeführte Kehlkopf-Extirpation und die Anwendung eines Künstlichen Kehlkopfes. *Arch. Clin. Chir.* **17**:343-356.

Hansen RH, Schonhorn (1966), A new technique for preparing low surface energy polymers for adhesive bonding. *J. Polym. Sci., Polym. Lett. Ed.* **B4**:203-209.

Harkes G, Feijen J., Dankert J (1991), Adhesion of *Escherichia coli* on to a series of poly(methacrylates) differing in charge and hydrophobicity. *Biomaterials* **12**:853-860.

Herrmann IF, Kley W (1981), Die Glottoplastik mit einer Ventil-Prothese. *Arch. Otorhinolaryngol.* **231**:647.

Herrmann IF, Poschet G, Zöhren J (1986), Wear and tear on the silicon of valve prostheses in the upper digestive

Herrmann IF, Poschet G, Zöhren J (1986), Wear and tear on the silicon of valve prostheses in the upper digestive tract. A study using electron microscope scanning. In: Speech restoration via voice prostheses. Ed: IF Herrmann. Springer Verlag, Berlin-Heidelberg, p 69-73.

Hilgers FJM, Schouwenburg PF (1990), A new low-resistance, self-retaining prosthesis (Provox®) for voice rehabilitation after total laryngectomy. *Laryngoscope.* **100**:1202-1207.

Hilgers FJM, Balm AJM (1993), Long term results of vocal rehabilitation after total laryngectomy with the low-resistance, indwelling Provox® voice prosthesis system. *Clin. Otolaryngol.* **18**:517-523.

Hollahan JR, Bell AT (1974), Techniques and applications of plasma chemistry. Hollahan JR and Bell AT (Eds). John Wiley, New York.

Ho CP, Yasuda H (1988), Ultrathin coating of plasma polymer of methane applied on the surface of silicone contact lenses. *J. Biomed. Mat. Res.* **22**:919-937.

Hsieh YL, Timm DA, Wu M (1989), Solvent and glow discharge induced surface wetting and morphological charges of poly(ethylene terephthalate) (PET). *J. Appl. Polym. Sci.* **38**:1719-1737.

Hsiue GH, Lee SD, Wang CC, Shiue MHI, Chang PCT (1994), Plasma-induced graft copolymerisation of HEMA onto silicone rubber and TPX film inproving rabbit corneal epithelial cell attachment and growth. *Biomaterials* **15**:163-171.

Izdebski K, Ross JC, Lee S (1987), Fungal colonization of tracheoesophageal voice prostheses. *Laryngoscope.* 97:594-597.

Jeon SI, Andrade JD (1991), Protein-surface interactions the presence of polyethylene oxide: II. Effect of protein size. *J. Colloid Interf. Sci.* **142**:159-166.

Jolley JG, Geesey GG, Hankins MR, Wright RB, Wichlacz PL (1988), Auger electron spectroscopy and X-ray photoelectron spectroscopy of the biocorrosion of copper by gum arabic, bacterial culture supernatant and *Pseudomonas atlantica* exopolymer. *Surface Interface Anal.* **11**:371-376.

Kalman PG, Ward CA, McKeown NB, McCullough D, Romaschin AD (1991), Improved biocompatability of silicone rubber by removal of surface entrapped air nuclei. *J. Biomed. Mat. Res.* **25**:199-211.

Kristinsson K (1989), Adherence of staphylococci to intravascular catheters. *J. Med. Microbiol.* **28**:249-257.

Lai JY, Lin YY, Denq YL, Shyu SS, Chen JK (1996), Surface modification of silicone rubber by gas plasma treatment. *J. Adhesion Sci. Technol.* **10**:231-242.

Lappin-Scott HM, Costerton JW (1989), Bacterial biofilms and surface fouling. *Biofouling.* **1**:323-342.

Lee JH, Park JW, Lee HB (1991), Cell adhesion and growth on polymer surfaces with hydroxyl groups prepared by water vapour plasma treatment. *Biomaterials* **12**:443-448.

Liston EM, Martinu L, WertheimerMR (1993), Plasma surface modification of polymers for improved adhesion: a critical review. *J. Adhesion Sci. Technol.* 7:1091-1127.

Mahieu HF, Van Saene JJM, Den Besten J (1986), Oropharynx decontamination preventing Candida vegetation on voice prostheses. *Arch. Otolaryngol. Head Neck Surg.* **112**:1090-1092.

Mahieu HF, Annyas AA, Schutte HK, Van der Jagt EJ (1987), Pharyngoesophageal myotomy for vocal rehabilitation of laryngectomies. *Laryngoscope.* 97:451-457.

Mahieu HF (1988), Voice and speech rehabilitation following laryngectomy. Ph-D Thesis, University of Groningen, Groningen, The Netherlands. pp 178.

Marsh PD, Martin MV (1992), Oral microbiology. 3nb rev. ed. London: Chapman and Hall.

Modica LA (1987), Care of tracheo-esophageal voices prostheses (letters to the editor). *Arch. Otolaryngol. Head Neck Surg.* **113**:436-437.

Morra M, Occhiello E, Garbassi F (1990), Surface characterization of plasma-treated PTFE. *Surface Interface Anal.* **16**:412-417.

Natarajan B, Richardson MD, Path MRC, Irvine BWH, Thomas M (1994), The Provox™ voice prosthesis and Candida albicans growth: a preliminary report of clinical, mycological and scanning electron microscopic assessment. *J Laryngology and Otology.* **108**:666-668.

Neu TR, Marshall KC (1990), Bacterial polymers: physico-chemical aspects of their interactions at interfaces. *J. Biomater. Appl.* **5**:107-133.

Neu TR, van der Mei HC, Busscher HJ, Dijk F, Verkerke GJ (1993), Biodeterioration of medical-grade silicone rubber used for voice prostheses: a SEM study. *Biomaterials.* **14**:459-464.

Neu TR, Verkerke GJ, Herrmann IF, Schutte HK, van der Mei HC, Busscher HJ (1994), Microflora on explanted silicone rubber voice prostheses: taxonomy, hydrophobicity and electrophoretic mobility. *J. Appl. Bacteriology.* **76**:521-528.

Neu TR, de Boer CE, Verkerke GJ, Schutte HK, Rakhorst G, van der Mei HC, Busscher HJ (1994), Biofilm development in time on a silicone voice prosthesis, a case study. *Microb. Ecol. in Health and Disease.* **7**:27-33.

Neumann AW, Hum OS, Francis DW, Zingg W, van Oss CJ (1980), Kinetic and thermodynamic aspects of platelet adhesion from suspension to various substrates, *J. Biomed. Mat. Res.* **14**:499-509.

Nichols MF, Hahn AW, Easley JR, Mayhan KG (1979), Bioevaluation of plasma polymerized films in skeletal muscle. *J. Biomed. Mater. Sci.* **13**:299-315.

Nickel JC, Ruseska I, Wright JB, Costerton JW (1985), Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. *Antimicrobial Agents and Chemotherapy* **27**:619-624.

Nijdam HF, Annyas AA, Schutte HK, Leever H (1982), A new prosthesis for voice rehabilitation after laryngectomy. *Arch. Otorhinolaryngol.* **237**:27-33.

Nijdam HF, Manni JJ, and Nicolasen M (1990), A new valveless low-pressure voice prostheses. *Clin. Otolaryngol.* **15**:561.

Olsson J, Glantz PO, Krasse B (1976), Surface potential and adherence of oral streptococci to solid surfaces. *Scan. J. Dent. Res.* **84**:240-242.

Owens DK, Wendt RC (1969), Estimation of the surface free energy of solids. *J. Appl. Polymer Sci.* **13**:1741.

Owen MJ, Smith PJ (1994), Plasma treatment of polydimethylsiloxane. *J. Adhesion Sci. Technol.* **8**:1065-1075.

Palmer MD, Johnson AP, Elliott TSJ (1993), Microbial colonization of Blom-Singer prostheses in postlaryngectomy patients. *Laryngoscope.* **103**:910-914.

Pashley RM, Israelachvili JN (1984), DLVO and hydratation forces between mica surfaces in Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} chloride solutions. *J. Coll. Interf. Sci.* 97:446-455.

Ratner BD (1993), New ideas in biomaterials science; a path to engineered biomaterials. *J. Biomed. Mat. Res.* **27**:837-850.

Rutter PR, Vincent B (1980), The adhesion of microorganisms to surfaces: physico-chemical aspects. In: Microbial Adhesion to surfaces, Berkeley RCW, Lynch JM, Melling J, Rutter PR, Vincent B (Eds), Ellis Horwood Ltd., Chischester, England. pp 79-93.

Schierholz J, Jansen B, Jaenicke L, Pulverer G (1994), In-vitro efficacity of an antibiotic releasing silicone ventricle catheter to prevent shunt infection. *Biomaterials* **15**:996-1000.

Singer MI, Blom ED (1981), Selective myotomy for voice restoration after total laryngectomy. *Arch. Otolaryngol.* **107**:670-673.

Staffieri M, Staffieri A (1988), A new voice button for post-total laryngectomy speech rehabilitation. *Laryngoscope.* **98**:1027-1029.

Sutherland IW (1977), Bacterial exopolysaccharides, their nature and production. In: Surface Carbohydrates of the Prokaryotic Cell. Sutherland IW (Ed). Academic press, London, New-York, San-Fransisco. pp. 27-96.

Traissac L, Devars F, Gioux M, Petit J, Benjebria A, Henry CH, Ucelay I (1987), La réhabilitation vocale du laryngectomisé par implant phonatoire. *Revue de laryngologie* **108**:157-159.

Van den Hoogen FJA, Oudes MJ, Janssen P, Hombergen G, Nijdam HF, Manni JJ (1996), The Groningen, Nijdam and Provox voice prostheses: a prospective clinical comparison based on 845 replacement. *Acta. Otolaryngol. (Stockh)* **116**:119-124.

Van Loosdrecht MCM, Lyklema J, Norde W, Zehnder AJB (1990), Influences of interfaces on microbial activity. *Microbiol. Rev.* **54**:75-87.

Van Oss CJ, Good RJ, Chaudhury MK (1986), The role of van der Waals forces and hydrogen bonds in hydrophobic interactions between biopolymers and low energy surfaces. *J. Coll. Interf. Sci.* **11**:378-390.

Van Oss CJ, Good RJ, Chaudhury MK (1988), Additive and nonadditive surface tension components and the interpretation of contact angles. *Langmuir* **4**:884-891.

Van Wachem PB, Beugeling T, Feijen J, Bantjes A, Detmers JP, van Aken (1985), Interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities. *Biomaterials* 6:403-408.

Van Weissenbruch R, Bouckaert S, Remon J-P, Nelis HJ, Aerts R, Albers FWJ (1997), Chemoprophylaxis of fungal deterioration of the Provox prosthesis. *Ann. Otol. Rhinol. Laryngol.* in press.

Wimpenny JWT (1994), The spatial organisation of biofilm. in: Bacterial biofilms and their control in medicine and industry. Wimpenny J, Nichols W, Stickler D, Lappin-Scott H (Eds), BioLine, Antony Rowe Ltd, Chippenham, Wiltshire. UK.

Wood J, Sharma R (1995), How long is the long-range hydrophobic attraction? *Langmuir* **11**:4797-4802.

Yasuda H (1985), in: Plasma Polymerization. Yasuda H (Ed), Academic Press Inc, Orlando.

HYDROPHOBIC RECOVERY OF REPEATEDLY PLASMA-TREATED SILICONE RUBBER. I. STORAGE IN AIR.

E.P. Everaert, H.C. van der Mei, J. de Vries, and H.J. Busscher*

ABSTRACT Silicone rubber is used for a wide variety of biomedical and industrial applications due to its good mechanical properties, combined with a hydrophobic surface. Frequently, however, it is desirable to alter the surface hydrophobicity of silicone rubber. Often this is done by plasma treatments but the effects are usually transient. In this study, surfaces of medical grade silicone rubber have been repeatedly modified by means of oxygen, argon, carbon dioxide, and ammonia RF plasma treatments with a 24 h time interval in between treatments. Treated samples were stored in air, prior to surface characterization by water contact angle measurements, X-ray photoelectron spectroscopy (XPS), streaming potential measurements and profilometry for surface roughness. The carbon percentage of the surfaces decreased after plasma treatment while the silicon and oxygen percentages increased irrespective of the plasma used. The formation of Si-O-Si bridges between siloxane chains after plasma treatment was demonstrated by the appearance of a new component in the Si_{2p} peak but the degree to which this occurred differed per gas. Streaming potential measurements in a 10 mM potassium phosphate buffer indicated a more negatively charged surface for treated samples compared to untreated samples (-23.3 mV at pH 7.0). Surface roughness increased slightly for repeatedly plasma-treated samples from R_A= 0.35 μm to R_A= 0.46 μm while scanning electron microscopy showed the presence of several "cracks" spanning the surface after repeated treatment. Argon, carbon dioxide and ammonia plasmas significantly reduced the advancing water contact angle from 115° to 58°, 72°, and 85°, respectively, on a more permanent basis (especially when the treatments were repeated after recovery). Oxygen plasma effects on water contact angles generally disappeared within 5 h, also after repeated treatment.

INTRODUCTION

Silicone polymers exhibit good mechanical properties for a variety of biomedical and industrial applications. For instance, silicone rubber has been used for voice prostheses (Mahieu, 1988; Neu *et al.*, 1993), urinary catheters (Farber and Wolff, 1993), contact lens materials (Holly and Owen, 1983), and icing coating material (Andersson *et al.*, 1994). However, their inherently high hydrophobicity limits certain applications of this material despite its favorable mechanical properties (Owen and

*With permission of VSP, J. Adhesion Sci. technol, 9: 1263-1278 (1995).

Smith, 1994). Plasma treatment of silicone polymers may affect their hydrophobicity, and thereby their bondability to other materials, without affecting the bulk properties. Plasma treatment often involves progressive oxidation of the surface and crosslinking of surface molecular groups, which inhibits the migration of low molecular weight oligomers from the bulk to the surface.

Various gases have been used to modify silicone polymers by plasma treatment, such as oxygen (Owen and Smith, 1994; Morra *et al.*, 1990), helium (Owen and Smith, 1994; Triolo and Andrade, 1983), carbon dioxide (Stewart and Urban, 1988), ammonia (Stewart and Urban, 1988), nitrogen (Owen and Smith, 1994; Gaboury and Urban, 1991) and argon (Owen and Smith, 1994; Stewart and Urban, 1988; Gaboury and Urban, 1991). Frequently a thin crosslinked, sometimes water-washable, silica-like surface layer was produced by plasma treatment, but there is no consensus about the nature of the chemical groups produced at the outermost surface. It has been suggested that the polar entities created might be silanol groups or other oxidized carbon species, e.g. aldehyde or carboxylic groups (Morra *et al.*, 1990).

The surface hydrophilicity created by plasma treatment is often lost over time (Owen and Smith, 1994; Morra *et al.*, 1990; Van der Mei *et al.*, 1991). This so-called hydrophobic recovery can be influenced by the storage conditions, whether in air or in liquid, temperature or subsequent adsorption of a surfactant (Van Dyke *et ak.*, 1991). A number of mechanisms for the hydrophobic recovery of plasma treated silicone rubber have been proposed by Owen and Smith (1994), including :

(1) reorientation of surface hydrophilic groups away from the surface;
(2) migration of treated polymer chains from the surface to the bulk;
(3) migration of untreated polymer chains from the bulk to the surface;
(4) loss of volatile oxygen-rich or other polar entities to the atmosphere;
(5) surface silanol condensation preventing chain reorientation;
(6) changes in surface roughness; and
(7) external contamination of the polymer surface.

Recently, Owen and Smith (1994) reported that the effects of RF treatments of a polydimethylsiloxane (PDMS) elastomer were broadly similar for argon, helium, oxygen and nitrogen. All treatments yielded a thin, brittle, silica-like layer on the surface as concluded from XPS and electron microscopy. Although no contact angles were measured in this study, which is necessary to determine directly the hydrophobic recovery of plasma-treated surfaces, it was suggested that hydrophobic recovery originated from migration of untreated polymer chains from the bulk to the surface through cracks in the silica-like layer.

Van der Mei *et al.* (1991), hypothesizing that a thick treated layer might inhibit the migration of hydrophobic groups towards the surface, reported that repeated oxygen plasma treatment of

polyethylene, with a 7-days interval in between, was more effective in creating a permanently hydrophilized surface than employing a higher RF power or a longer duration of the treatment.

In this study, we modified the surface of medical grade silicone rubber by repeated RF plasma treatments using various discharge gases including oxygen, argon, carbon dioxide, and ammonia in an attempt to create more permanent effects. The temporal behavior of the effects on the physico-chemical properties of the silicone rubber has been investigated using different surface characterization techniques, including water contact angle measurements, X-ray photoelectron spectroscopy (XPS), streaming potential measurements, profilometry (for surface roughness), and scanning electron microscopy.

EXPERIMENTAL

Materials

A Silastic Medical Grade Silicone Rubber (Q7-4750, Dow Corning) kit was purchased and 1-mm-thick 50x70 mm and 2.8-mm-thick 25x76 mm plates were produced following the procedures suggested by the manufacturer. Briefly, equal proportions of part A and part B were thoroughly blended together and injected into a mold at room temperature through a 3 mm diameter opening with a force of 3000 Kg. Subsequently, the silicone rubber was immediately cured at 200°C for 50 minutes. Finally, samples were cleaned in a 5 % RBS 35 (Omnilabo International B.V., The Netherlands) detergent solution under simultaneous sonication (5 minutes, 150 W) and thoroughly rinsed in Millipore grade water and absolute ethanol (>96 %).

Plasma treatment

The silicone rubber samples were repeatedly glow-discharged in a PLASMOD instrument (Tegal Corporation, Richmond, CA, USA). The PLASMOD is commercially available, inductively coupled (13.56 MHz RF) instrument equipped with a cylindrical quartz-made reaction chamber (inner diameter 8 cm, length 15 cm). Pumping down was done with a Balzers rotary pump (320 l/min) using a liquid nitrogen cold trap. All plasma treatments were done under 3.7 Torr gas pressure for 60 s and at a RF power of 50 W. Oxygen (99.5 %), argon (99.996 %), carbon dioxide (99.99 %) and ammonia (99.98 %) gases were obtained from Hoekloos Nederland B.V., The Netherlands.

The treated samples were stored in air in disposable Petri dishes and used immediately for water contact angle measurements, and after 1 month for XPS characterization. As the hydrophobic recovery of the treated silicone rubber occurred within a few hours, plasma treatments were repeated every 24 h. This cycle was repeated six times. Samples once used for surface characterization were not used again. All values given in this paper are the means of experiments on two separately prepared samples.

Contact angle measurements

Advancing and receding water contact angles were measured at room temperature with an image analyzing system, using the sessile drop technique. The advancing and receding angles were obtained by keeping the needle in the water droplet after positioning on the surface and by carefully moving the sample until the advancing angle appeared maximally. Each value was obtained by averaging results of at least ten droplets on one sample.

Elemental surface composition

X-ray photoelectron spectroscopy (XPS) was performed on each treated sample using a S-Probe spectrometer (Surface Science Instruments, Mountain View, CA, USA) equipped with an aluminum anode (10 kV, 22 mA) and a quartz monochromator. The direction of the photoelectron collection angle was 55° with the normal to the sample and the electron flood gun was set at 10 eV. A survey scan was made with a 1000x250 μm spot and a pass energy of 150 eV. Detailed scans of the C_{1s}, O_{1s}, N_{1s} and Si_{2p} lines were obtained using a pass energy of 50 eV. Binding energies were determined by setting the binding energy of the C_{1s} component due to carbon involved in siloxane (C-Si-O-Si) bonds at 284.5 eV (Beamson and Briggs, 1992). The experimental peaks were integrated after nonlinear background subtraction and the peaks were decomposed assuming a Gaussian/Lorentzian ratio of 85/15 by using the SSI PC software package. All Si_{2p} bands were fitted by fixing the distance between $Si_{2p(3/2)}$ and $Si_{2p(1/2)}$ at 0.7 eV and by imposing an intensity ratio of 2.0. Elemental surface compositions were expressed in atomic %, setting %C + %O + %Si + %N to 100%.

Surface roughness

The surface roughness R_A was determined on a Perthometer C5D (Perthen, Germany) equipped with a 2 μm stylus (opening angle of 90°). The R_A value indicates the average distance of the roughness profile to the center line of the profile. The R_A values reported were obtained by averaging ten scans on one sample. Furthermore, the influence of the plasma treatments on the surface topography was occasionally studied by scanning electron microscopy. To this end, the specimens were mounted on stubs and sputter-coated with gold (15 nm).

Streaming potential

Streaming potentials (V_{str}) in a 10 mM potassium phosphate solution (pH 3-8) were measured employing rectangular platinum electrodes (5.0 x 25.0 mm) located at both ends of a parallel plate flow chamber (Van Wagenen and Andrade). Two samples of silicone rubber (length 76 mm, width

25 mm, and thickness 2.8 mm), separated by a 0.2 mm Teflon gasket, constituted the top and bottom plates of the chamber.

Zeta potentials (ζ) were derived from the pressure dependence of the streaming potentials (V_{str}) measured, according to

$$\zeta = \frac{\eta}{\varepsilon} \cdot K_b \frac{\Delta V_{str}}{\Delta P} \qquad (1)$$

where ε is the dielectric permittivity, η is the viscosity and K_b is the solution conductivity. Note that the use of equation (1) implies that surface conduction is neglected. The conductivity of the electrolyte, K_b, was measured after each experiment, using a Knick Konduktometer 702 (Berlin, Germany). Measurements were done at ten different pressures ranging between 37.5 and 150 Torr and each pressure was applied for 10 s in both directions. The streaming potentials measured were independent of the flow direction and linearity between V_{str} and the applied pressure was always observed, at least for untreated silicone rubber.

RESULTS

Water contact angles

Fig. 1 shows examples of advancing and receding water contact angles on repeatedly treated silicone rubber in an argon and oxygen plasma as a function of the storage time and the number of plasma treatments. Immediately after each plasma treatment, the water contact angles showed a large decrease in surface hydrophobicity. As can be seen, however, all surfaces treated regained some degree of hydrophobicity after storage in air. As complete hydrophobic recovery of oxygen plasma-treated samples occurred within a few hours, water contact angles were measured at regular intervals during the storage period up to 320 min. An additional measurement was carried out after 24 h to observe the long-term recovery. In contrast to the hydrophobic recovery of oxygen plasma treated silicone rubber, argon plasma-treated samples did not show complete recovery.

Figs. 2 and 3 show the advancing and receding water contact angles, respectively, on silicone rubber repeatedly treated in oxygen, argon, carbon dioxide and ammonia plasmas as measured after different storage times. Oxygen plasma reduced the advancing water contact angles to only 60° after 5 min, but within 5 h the surface had regained its original hydrophobicity. Argon and carbon dioxide plasmas yielded a fully wettable surface after 1-5 treatments, while the hydrophobic recovery was only partial, even after 24 h. Note that for argon plasma, the degree of hydrophobic recovery decreased with the number of treatments. Ammonia plasma made a slightly wettable surface, with, however, only a minor hydrophobic recovery. From Figs. 2 and 3 it can be seen that advancing and receding contact

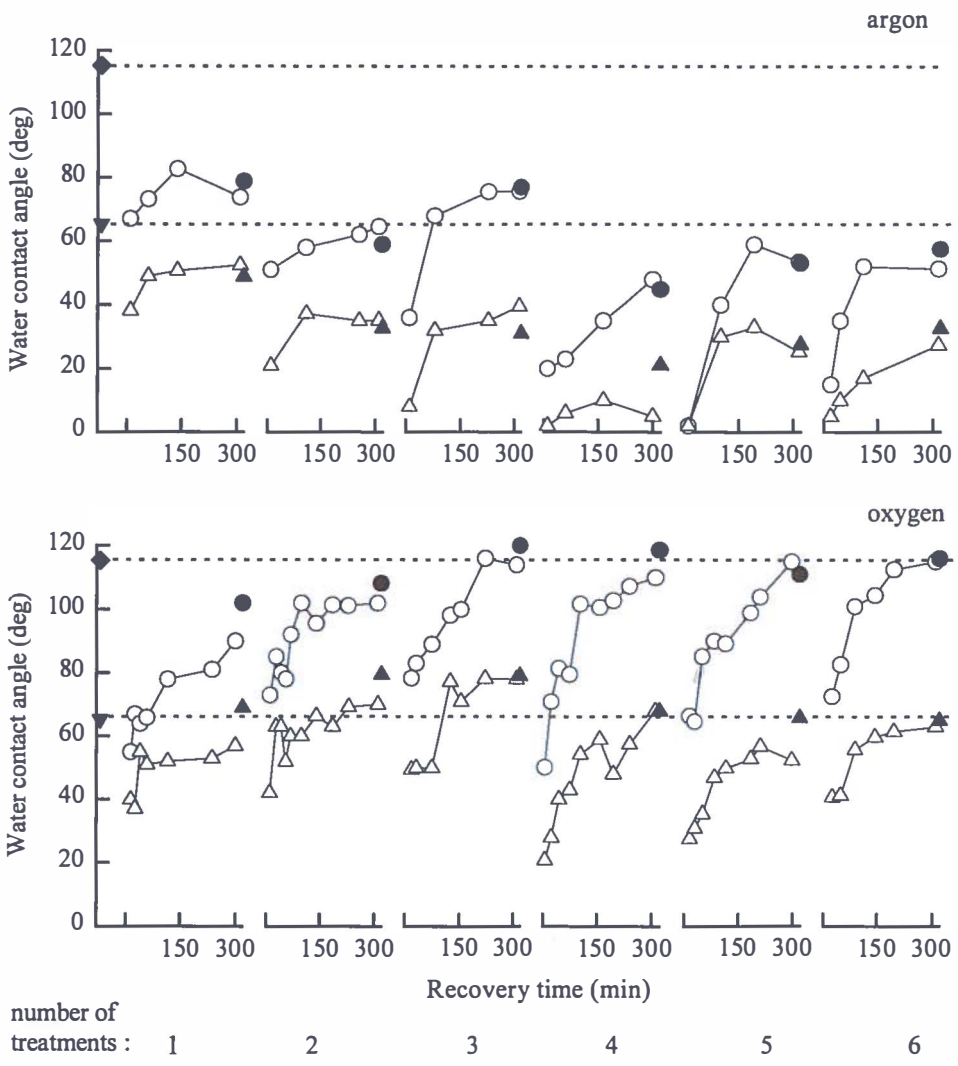


Figure 1. *Advancing (○) and receding (△) water contact angles (degrees) on repeatedly argon and oxygen plasma-treated silicone rubber (50 W, 3.7 Torr, 60 s) stored in air. The open symbols represent contact angles measured up to 320 min after treatment, while the filled symbols represent contact angles measured after 24 h of aging. The (◆) and (▼) symbols represent the advancing and the receding water contact angles for untreated silicone rubber. The standard deviation over ten measurements on one sample amounted to 3° on average, while results of two separately prepared samples generally coincided within 6°.*

angles on RF plasma treated-samples behaved almost the same in relation to their hydrophobic recovery, with a contact angle hysteresis of around 30° on average, which is low compared with the hysteresis on untreated silicone rubber (50°).

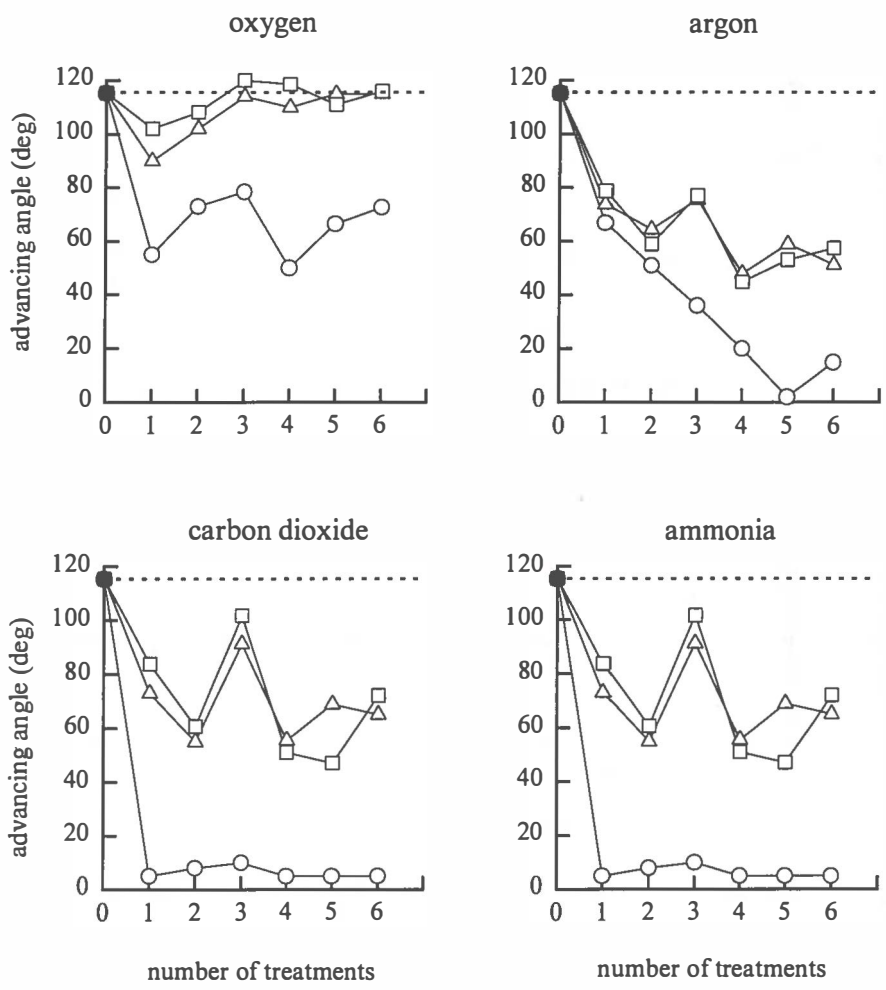


Figure 2. *Advancing water contact angles (degrees) on silicone rubber repeatedly plasma-treated in oxygen, argon, carbon dioxide and ammonia, after storage times of 5 min (○), 5 h (△) and 24 h (□). The (•) symbol represents the advancing contact angle for untreated silicone rubber. The standard deviation over ten measurements on one sample amounted to 3° on average, while the results of two separately prepared samples generally coincided within 6°.*

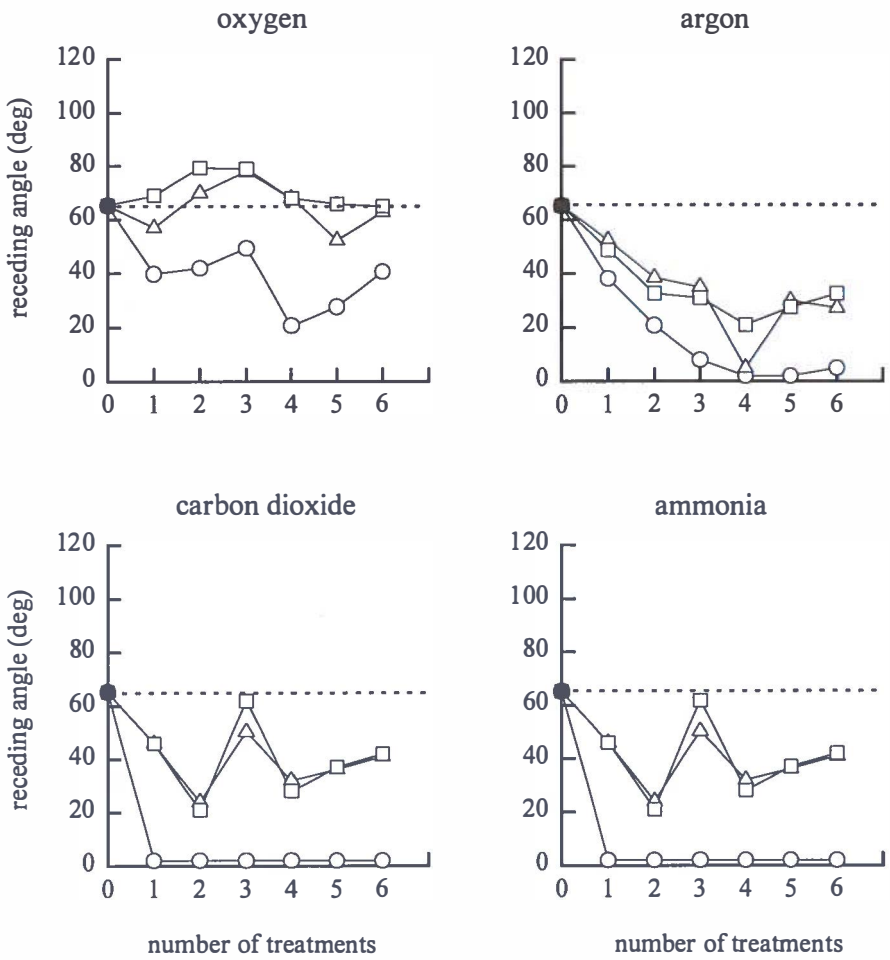


Figure 3. *Receding water contact angles (degrees) on silicone rubber repeatedly plasma-treated in oxygen, argon, carbon dioxide and ammonia, after storage times of 5 minutes (○), 5 hours (△) and 24 hours (□). The (•) symbol represents the receding contact angle for untreated silicone rubber. The standard deviation over ten measurements on one sample amounted to 3° on average, while the results of two separately prepared samples generally coincided within 6°.*

Elemental surface composition

Fig. 4 summarizes the elemental surface compositions by XPS for untreated and repeatedly plasma-treated silicone rubber. The carbon content decreased with increasing number of plasma treatments

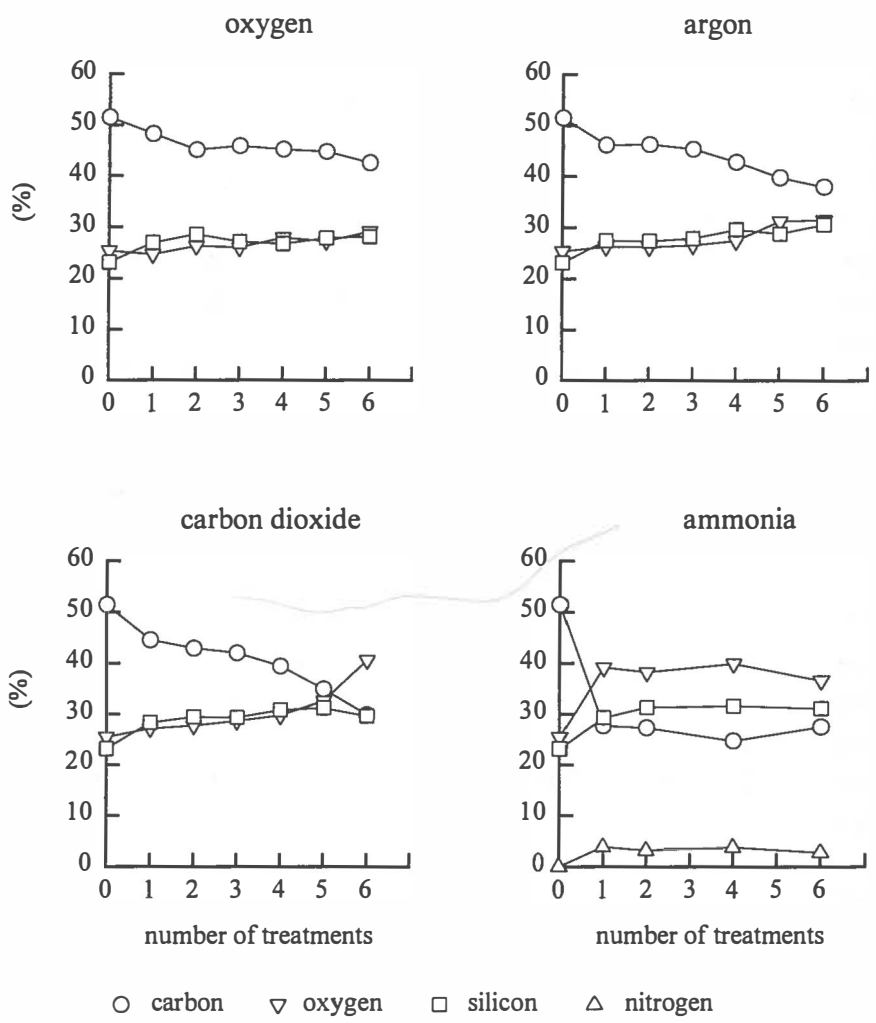


Figure 4. *Elemental surface composition of silicone rubber repeatedly treated with oxygen, argon, carbon dioxide, and ammonia plasmas (50 W, 3.7 Torr, 60 s) as a function of the number of treatments. The closed symbols represent the values for untreated silicone rubber. The standard deviation over three measurements on one sample amounted to 1 % on average for untreated silicone rubber, while results for two separately treated samples coincided within 3 %.*

while the silicon and the oxygen amounts increased, irrespective of the plasma used. The decrease in carbon content differed among the various gases used, from an initial value of 51.5 % to 42.6 % for oxygen, 38.0 % for argon, 29.8 % for carbon dioxide, and 27.5 % for ammonia. Nitrogen was not detected by XPS except for the ammonia-treated samples, for which the nitrogen content measured ranged from 2 to 4 %.

The C_{1s}, O_{1s}, and Si_{2p} peaks presented multiple components. Usually the C_{1s} peak had two components at 284.5 and 285.6 eV for carbon involved in methyl groups of the siloxane bond and in crosslinking between the siloxane chains, respectively. After 5-6 repeated plasma treatments, two additional C_{1s} components for carbon involved in oxygen bonds appeared at 287.6 and 289.8 eV. The O_{1s} peak had two components, one at 532.0 eV, due to the oxygen atoms in siloxane bonds, and the other at 533.2 eV, attributed to silanol groups and other Si-O bonds. The Si_{2p} peak showed a new component as well. Consequently, the $Si_{2p(3/2)}$ had a component at 101.9 eV indicative of the silicon in siloxane polymer chains and at 103.4 eV attributed to silicon in other multiple oxygen bonds, as in silica. In Fig. 5, it can be seen that the relative prevalence of the new Si_{2p} component increases with the number of plasma treatments, irrespective of the type of plasma used.

Surface roughness

The surface roughness increased slightly from its original smooth surface value R_A= 0.35 μm to approximately R_A= 0.46 μm after 6 repeated plasma treatments, independent of the type of plasma employed. However, scanning electron microscopy showed the presence of several "cracks" spanning the surface (see Fig. 6) which remained nevertheless undetected by profilometry and which were equally present on all treated samples.

Streaming potential

Fig. 7 shows the zeta potentials of untreated silicone rubber as a function of the pH. The zeta potential of untreated silicone rubber ranged from -24.2 mV at pH 8.0 to -2.9 mV at pH 3.0. For RF plasma-treated samples, a constant zeta potential could not be observed during application of a certain pressure (see also Fig. 8), but the values measured indicated that plasma-treated silicone rubber was up to 20 mV more negatively charged over the entire pH range than untreated silicone rubber.

DISCUSSION

Water contact angle

There is a major water contact angle hysteresis on both RF plasma treated and untreated silicone

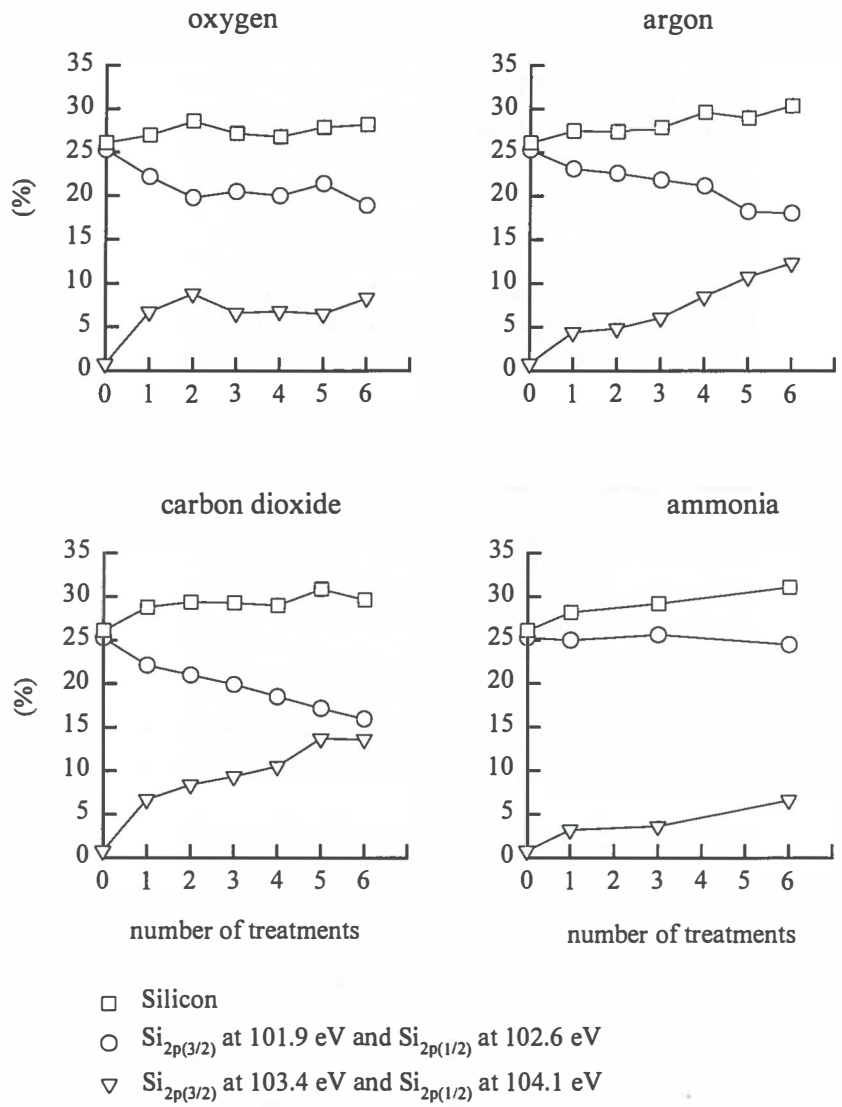


Figure 5. *Si_{2p} elemental surface concentration and Si_{2p} components of untreated (filled symbols) silicone rubber and of oxygen, argon, carbon dioxide and ammonia plasma-treated silicone rubber (open symbols) at 50 W, 3.7 Torr, 60 s and stored in air as a function of the number of plasma treatments. The standard deviation over three measurements on one sample amounted to 1% on average for untreated silicone rubber, while results for two separately treated samples coincided within 3%.*

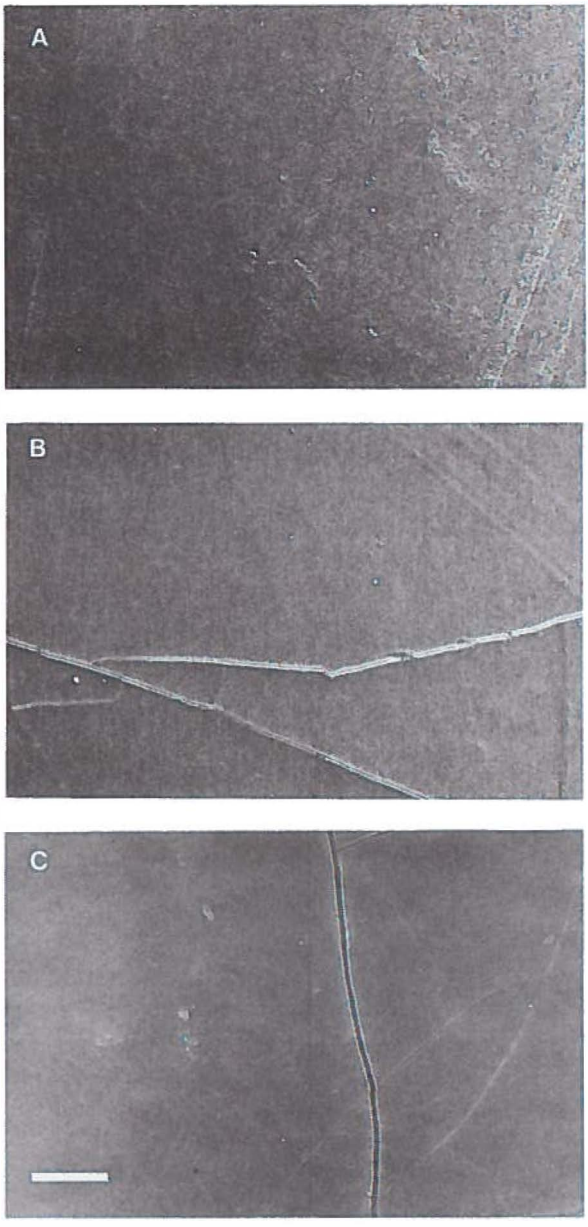


Figure 6. *Scanning electron micrographs of (A) untreated silicone rubber, (B) silicone rubber treated six times in an argon plasma with a 24 h recovery period in between each treatment, and (C) silicone rubber treated six times in an oxygen plasma with a 24 h recovery period in between each treatment. The bar equals 10.0 μm.*

rubbers, despite the fact that the surface roughness is in the submicrometer range. Therefore (Busscher et al., 1984), it is likely that this hysteresis is related to the high flexibility of the siloxane backbone of the elastomer.

It has been reported (Owen, 1988) that the rotational freedom of the siloxane backbone allows the exposure of the methyl groups to their best effect, i.e. away from the surface in water and towards the surface in air. Consequently, this yields a relatively low receding water contact angle and a high advancing contact angle. The high mobility of polymer chains in silicone rubber may also explain why the hydrophobic recovery of plasma-treated samples occurs in several hours. By comparison, van der Mei *et al.* (1991) reported that hydrophobic recovery of repeated oxygen plasma-treated polyethylene was seen in more than 8 days. Obviously, the mobility of untreated polyethylene chains from the bulk to the surface is much slower than that of untreated siloxane polymer chains, in accordance with the difference in glass transition temperatures of polyethylene (-35°C) and siloxane polymer chains (-125°C).

It is unclear why the kinetics of hydrophobic recovery differs for the various gases used. Although cracks have been suggested as a starting point for hydrophobic recovery by migration of untreated chains to the surface (Owen and Smith, 1994), no significantly different amounts of cracks were observed for silicone rubber samples treated with different gases. Possibly, the thickness, density and composition of the argon and carbon dioxide plasma-treated silicone rubber samples allows less migration of untreated chains than when oxygen or ammonia plasmas are employed.

Generally, the water contact angle hysteresis on RF plasma-treated silicone rubber was smaller than that of untreated silicone rubber. This may be due to a loss of the rotational freedom of the treated polymer chains. Such loss can be caused by formation of new chemical groups and cross-linking, as detected by XPS.

Elemental surface composition

The elemental surface composition of our untreated silicone rubber is identical to the surface composition of PDMS found by Owen and Smith (1994) and close to the values found by Ratner *et al.* (1993) and by Raimondi *et al.* (1995). The general trends of the elemental surface composition changes observed (Fig. 4) upon RF plasma treatment are similar to that reported by Owen and Smith (1994). Although they concluded that for single plasma treatment the effects were similar for four different gases used, we found that the degree of hydrophobic recovery, as revealed also by XPS, differs per gas (see Figs. 4 and 5).

After single plasma treatment, new components in the C_{1s}, O_{1s} and Si_{2p} peaks appeared, as are also formed after repeated treatments (Owen and Smith, 1994). Interestingly, the O_{1s} component at

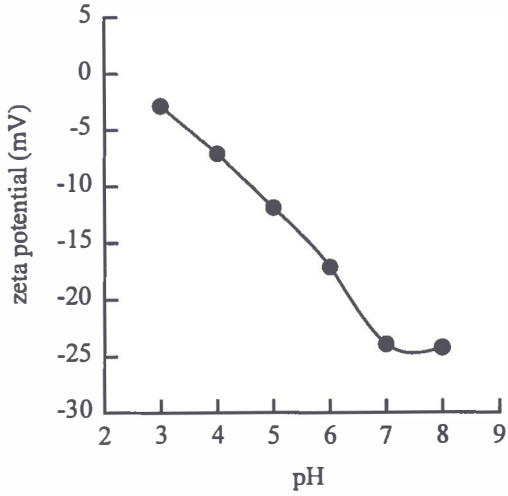


Figure 7. *Zeta potentials in a 10 mM potassium phosphate solution of untreated silicone rubber as a function of the pH and measured by streaming potentials. The results of two separately prepared samples coincided within 3 mV.*

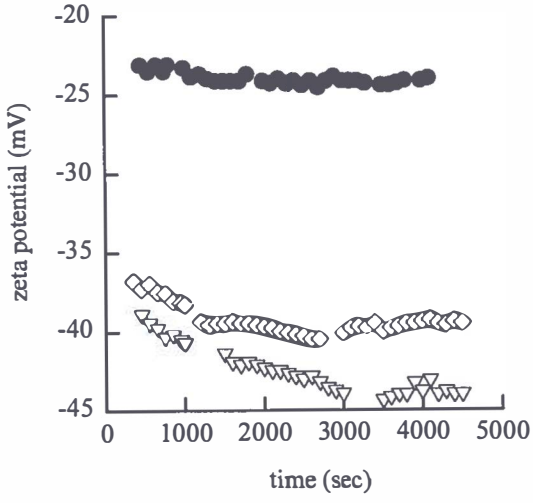


Figure 8. *Zeta potentials in a 10 mM potassium phosphate buffer (pH 7.0) of untreated silicone rubber (•) and of argon (◇) and carbon dioxide (▽) plasma-treated silicone rubber as a function of the time of application of a pressure of 112.5 Torr alternating in both directions. The values of two separately prepared samples coincided within 4 mV.*

533.2 eV (attributed to the oxygen included in new Si-O-Si or silica-like bonds) correlated linearly with the new Si_{2p} component with a slope that varies per gas (see Fig. 9). This suggests that new Si-O-Si bounds have been formed at the treated surface, by condensation of silanol groups (Hollahan and Carlson, 1970) leading to bridging between siloxane chains, but that the extent to which this occurs differs per gas.

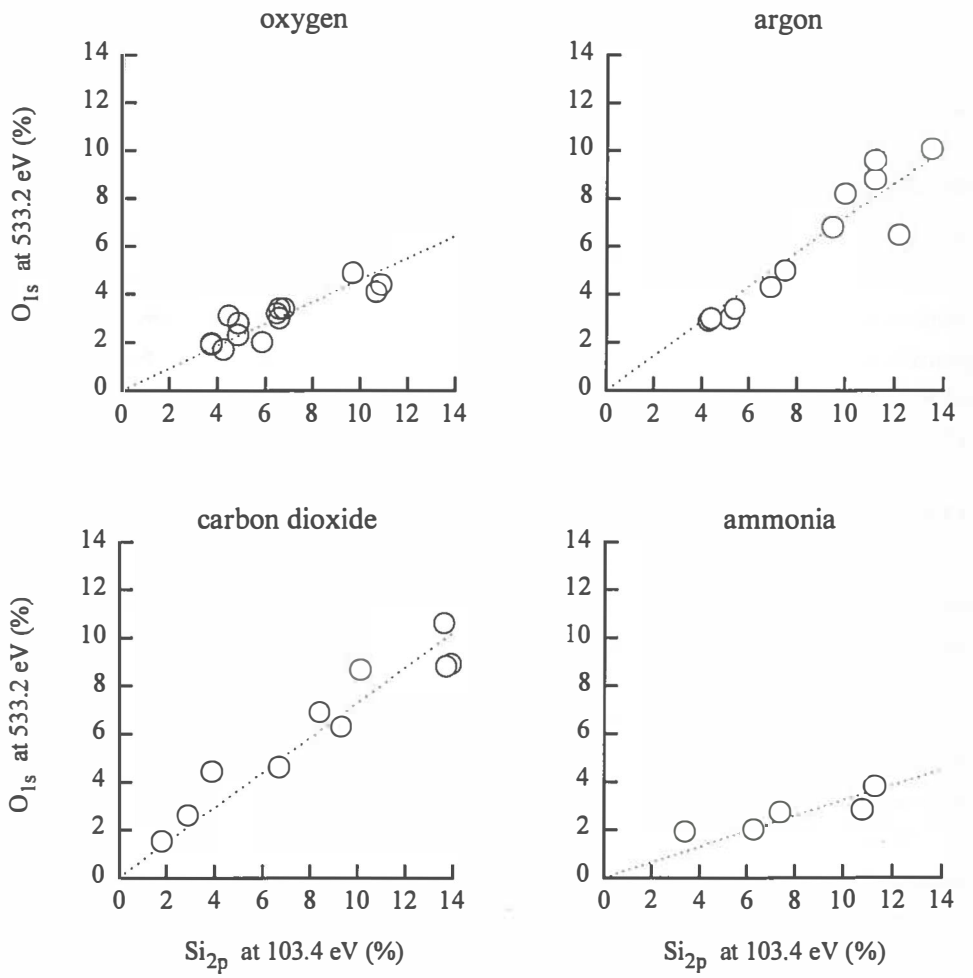


Figure 9. *The component of the O_{1s} peak at 533.2 eV as a function of the new Si_{2p} component at 103.4 eV of oxygen, argon, carbon dioxide and ammonia plasma-treated silicone rubber. Linear regression analyses of data yielded the dotted lines, with slopes of 0.46, 0.72, 0.73 and 0.32 for oxygen, argon, carbon dioxide and ammonia, respectively.*

Surface roughness

The effects of plasma treatments on the R_A values of repeatedly RF plasma-treated silicone rubber are minor, irrespective of the gas used. On average, after six repeated treatments, R_A increased slightly by 0.1 μm, which is not enough to account for the changes in contact angle hysteresis encountered. Obviously occasional cracks are not reflected in the R_A values. Although cracks spanning RF plasma-treated silicone rubber surfaces have been suggested to be a starting point for migration mechanisms during hydrophobic recovery (Owen and Smith, 1994), this suggestion is likely inadequate for explaining why the time scale of hydrophobic recovery differs per gas used.

Streaming potentials

The zeta potentials of untreated silicone rubber as derived from streaming potentials under the conditions employed are negative. No stable zeta potentials could be measured for the plasma-treated surfaces, possibly because the surface layers on silicone rubber created by RF plasma treatment are partially water-washable. Although not pursued any further, the change in time of streaming potentials of RF plasma treated silicone rubber during measurement might be a useful tool for studying the exact physico-chemical nature of the layer.

CONCLUSION

Single RF plasma treatment of silicone rubber usually yields transient effects due to, amongst others, the high mobility of the siloxane backbone and the migration of untreated polymer chains from the bulk to the surface during hydrophobic recovery. Also, the effects are described as being similar for different gases (Owen and Smith, 1994).

This study shows that hydrophobic recovery of silicone rubber after RF plasma treatment, can be slowed down considerably by repeating the treatment whereas the kinetics of hydrophobic recovery after repeated plasma treatment differs per gas, indicating that there is an effect of the type of gas.

No evidence was found that difference in the degree of cracking of surfaces treated by various gases could account for the difference in the degree of hydrophobic recovery observed. Thus, it is suggested that the various gases used for plasma treatment of silicone rubber surfaces yield top layers on the silicone rubber with different physico-chemical properties.

To analyze further the top layer created by plasma-treatment, we examined the top layer of ammonia plasma treated silicone rubber by angle-resolved XPS. Ammonia was chosen for this single-run experiment as it yielded incorporation of nitrogen, an element normally not found in silicone rubber. As presence of carbon contamination on surfaces often obscures the interpretation of angle-resolved XPS data, untreated silicone rubber was also examined as a control. Fig. 10 shows that

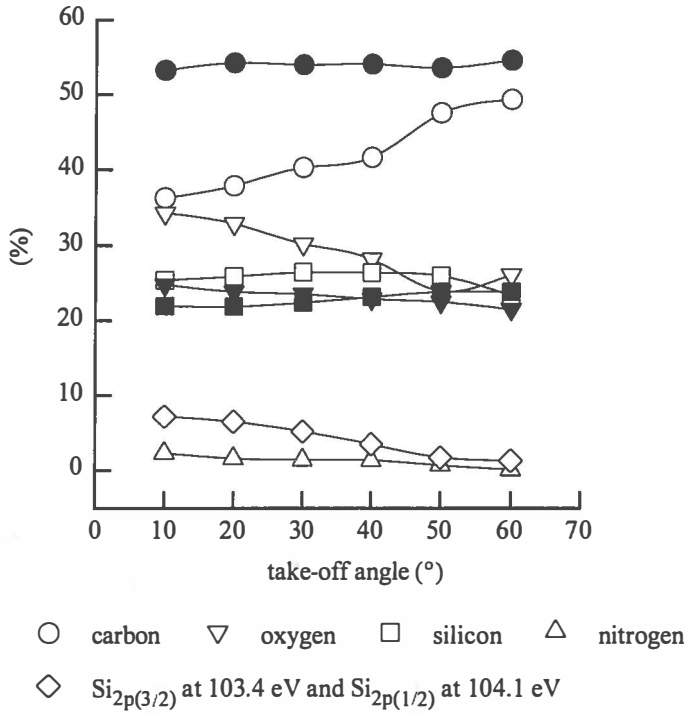


Figure 10. *Elemental surface composition of untreated silicone rubber and ammonia RF plasma treated silicone rubber (50 W, 3.7 Torr, 60 s) as a function of the electron take-off angle. The closed symbols represent the values for untreated silicone rubber.*

the composition of untreated silicone rubber was similar over the range of take-off angles employed, whereas a clear accumulation of elements due to the plasma treatment in the deeper surface layers can be seen. Interestingly, also the relative prevalence of the new component in the Si_{2p} peak indicative of the new Si-O-Si or silica-like bonds is most pronounced in the deeper surface layer (lowest take-off angle). These data can be taken as evidence to support the hypothesis that hydrophobic recovery is due to migration of untreated chains from the bulk to the surface.

REFERENCES

Andersson LO, Golander CL and Persson S (1994), Ice adhesion to rubber materials. *J. Adhesion Sci. Technol.* **8**:117-132.

Beamson G and Briggs D (Eds) (1992), In: High Resolution XPS of Organic Polymers The Scienta ESCA 300 Database, John Wiley & Sons, Chichester, pp. 268-269.

Busscher HJ, van Pelt AWJ, de Boer P, de Jong HP and Arends J (1984), The effect of surface roughening of polymers on measured contact angles of liquids. *Colloids Surfaces* 9:319-331.

Farber BF and Wolff AG (1993), Salicylic acid prevents the adherence of bacteria and yeast to silastic catheters. *J. Biomed. Mater. Res.* **27**:599-602.

Gaboury SR and Urban MW (1991), Spectrocopic evidence for Si-H formation during microwave plasma modification of poly(dimethylsiloxane) elastomer surfaces. *Polym. Communications* **32**(13):390-392.

Hollahan JR and Carlson GL (1970), Hydroxylation of polymethylsiloxane surfaces by oxidizing plasmas. *J. Appl. Polym. Sci.* **14**:2499-2508.

Holly FJ and Owen MJ (1983), In: Physicochemical Aspects of Polymer Surfaces, K.L. Mittal (Ed.), vol. 2, Plenum Press, New York. pp 625-636.

Mahieu HF (1988), Voice and speech rehabilitation following laryngectomy. Ph-D thesis, University of Groningen, Groningen, The Netherlands, pp 178.

Morra M, Occhiello E, Marola R, Garbassi F, Humphrey P and Johnson D (1990), On the aging of oxygen plasma-treated polydimethylsiloxane surfaces. *J. Colloid Interface Sci.* **137**:11-24.

Neu TR, van der Mei HC, Busscher HJ, Dijk F and Verkerke GJ (1993), Biodeterioration of medical-grade silicone rubber used for voice prostheses: a SEM study. *Biomaterials* **14**:459-464.

Owen MJ (1988), Surface tension of polytrifluoropropylmethylsiloxane. *J. Appl. Polym. Sci.* **35**:895-901.

Owen MJ and Smith PJ (1994), Plasma treatment of polydimethylsiloxane. *J. Adhesion Sci. Technol.* **8**:1063-1075.

Raimondi ML, Sassara C, Bellobono IR and Matturri L (1995), Kinetic study of release of silicon compounds from polysiloxane tissue expanders. *J. Biomed. Mater. Res.* **29**:59-63.

Ratner BD, Leach-ScampaviaD and Castner DG (1993), ESCA surface characterizationof four IUPAC reference polymers. *Biomaterials* **14**:148-152.

Stewart MT and Urban MW (1988), *Polym. Mater. Sci. Eng.* **59**:334-338.

Triolo PM and Andrade JD (1983), Surface modification and evaluation of some commonly used catheter materials. I. Surfaces properties. *J. Biomed. Mater. Res.* **17**:129-147.

Van der Mei HC, Stokroos I, Schakenraad JM and Busscher HJ (1991), Aging effects of repeatedly glow-discharged polyethylene: influence on contact angle, infrared absorption, elemental surface composition, and surface topography. *J. Adhesion Sci. Technol.* **5**:757-769.

Van Dyke MI, Lee H and Trevors JT (1991), Applications of microbial surfactants. *Biotechnology Adv.* 9:241-252.

Van Wagenen RA and Andrade JD (1980), Flat plate streaming potential investigations: hydrodynamics and electrokinetic equivalency. *J. Colloid Interface Sci.* **76**:305-314.

HYDROPHOBIC RECOVERY OF REPEATEDLY PLASMA-TREATED SILICONE RUBBER. 2. A COMPARISON OF THE HYDROPHOBIC RECOVERY IN AIR, WATER, OR LIQUID NITROGEN

E.P. Everaert, H.C. van der Mei, and H.J. Busscher*

ABSTRACT Surfaces of medical grade silicone rubber (Q7-4750, Dow Corning) were modified by repeated (six times) RF plasma treatments using various discharge gases: oxygen, argon, carbon dioxide and ammonia. The treated samples were stored for a period of 3 months in ambient air, water, or liquid nitrogen. Subsequently, the temporal behavior of the effects of the plasma treatment on the physico-chemical surface properties of the silicone rubber was investigated using water contact angle measurements and X-ray photoelectron spectroscopy (XPS). Hydrophobic recovery during 3 months storage in ambient air was considerable and nearly complete for all four plasmas used. Hydrophobic recovery was almost completely suppressed during storage in liquid nitrogen, and only a minor increase of around 10° in advancing water contact angle was observed for all four plasma treatments. Also during storage of treated samples in water, hydrophobic recovery was minimal and initiated again by returning the treated samples to ambient air. XPS analyses showed that argon, carbon dioxide and ammonia plasma-treated silicone rubber all had increased carbon percentages at the expense of oxygen and silicon after storage in water, or in liquid nitrogen, compared with after storage in ambient air. Interestingly, the carbon content of oxygen plasma-treated silicone rubber decreased during storage in water, or in liquid nitrogen, compared with storage in ambient air, while its oxygen and silicon percentages increased.

INTRODUCTION

Plasma treatments are well known to improve the wettability of polymer surfaces (Strobel *et al.*, 1994). However, because plasma-treated surfaces are frequently far from stable, the surface hydrophilicity created by the plasma treatment is often lost over time. This so-called "hydrophobic recovery" is caused, amongst others (Morra *et al.*, 1989; Garbassi *et al.*, 1989; Owen *et al.*, 1988, Owen and Smith, 1994; Triolo and Andrade, 1983; Foerch *et al.*, 1993; Strobel *et al.*, 1989), by the mobility and reorientation of polymer chains in the treated surface layer. Clearly, storage conditions such as temperature and the hydrophobicity/hydrophilicity of the storage medium (i.e. in air, or in water) will affect the kinetics and the final degree of hydrophobic recovery of a plasma-treated

*With permission of VSP. J. Adhesion Sci. Technol. 10: 351-359 (1996).

polymer surface. Previously, van der Mei *et al.* (1991) reported that the hydrophobic recovery of oxygen plasma-treated polyethylene was suppressed when the samples were stored in liquid nitrogen.

The inherently high hydrophobicity of silicone polymers limits certain applications of these materials, despite their favorable mechanical properties (Owen and Smith, 1994; Andersson *et al.*, 1994). Plasma treatment of silicone polymers (Everaert *et al.*, 1995; Owen and Smith, 1994; Triolo and Andrade, 1983; Andersson *et al.*, 1994; Stewart and Urban, 1990; Gaboury and Urban, 1990; Hollahan and Carlson, 1970) may affect their hydrophobicity and thereby their bondability to other materials, without affecting the bulk properties. Recently, Owen and Smith (1994) reported that the effects of RF discharge treatments of a polydimethylsiloxane (PDMS) elastomer were broadly similar for argon, helium, oxygen and nitrogen plasmas. All treatments yielded a thin, brittle, silica-like layer on the surface, as concluded from X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy. Although no contact angles were measured in this study, necessary to determine directly the hydrophobic recovery of plasma-treated surfaces, it was suggested that the hydrophobic recovery originated from the migration of untreated polymer chains from the bulk to the surface through cracks in the silica-like layer.

For polyethylene, showing hydrophobic recovery over a time scale of several days when stored in air, hydrophobic recovery could be decreased by repeating the plasma treatment (Van der Mei *et al.*, 1991). Repeated plasma treatment was suggested to yield a thicker treated layer through which fewer untreated chains could migrate to the surface. Silicone rubber after plasma treatment showed a hydrophobic recovery over a time scale of several hours (Everaert *et al.*, 1995) when stored in air, as opposed to the long-term recovery of polyethylene. However, repeated plasma treatment of silicone rubber with a 1-day time interval in between also yielded more stable, hydrophilic silicone rubber surfaces, especially after argon treatment. Contrary to the findings of Owen and Smith (1994), oxygen, argon, carbon dioxide and ammonia plasma-treated silicone rubber all had their own characteristic kinetics of hydrophobic recovery, indicating that different types of plasma yield treated layers of different thicknesses, densities, crosslinking and compositions (Everaert *et al.*, 1995).

The transient nature of plasma treatments of polymers greatly limits their applications. Often in, for example, packaging, it is possible to treat the surfaces by a plasma on a production line, but in many biomedical applications, such as for implants or contact lenses, it is not possible to treat the device immediately prior to implantation. Therefore, it remains an important challenge in materials science to minimize the hydrophobic recovery of plasma-treated materials as much as possible.

Whereas before (Everaert *et al.*, 1995) we modified the surface of medical grade silicone rubber by repeated RF plasma treatments using various discharge gases including oxygen, argon, carbon dioxide, and ammonia, it was the aim of this study to compare the influence of storage conditions on the hydrophobic recovery of repeatedly RF plasma-treated silicone rubber. Repeatedly

plasma-treated silicone rubber was stored for a period of 3 months in ambient air, water, or in liquid nitrogen. The temporal behavior of the effects of the plasma treatment on the physico-chemical properties of the silicone rubber were investigated using water contact angle measurements and XPS.

EXPERIMENTAL

Materials

Plates (1.0 mm thick) of Silastic Medical Grade Silicone Rubber (Q7-4750, Dow Corning) were produced following the procedures suggested by the manufacturer, as described before (Everaert *et al.*, 1995). Samples were cleaned in a 5 % RBS 35 (Omnilabo International B.V., Breda, The Netherlands) detergent solution under simultaneous sonication (5 min, 150 W) and thoroughly rinsed in Millipore grade water and absolute ethanol.

Plasma treatment

The silicone rubber samples were repeatedly plasma-treated in an inductively coupled (13.56 MHz RF) PLASMOD instrument (Tegal Corporation, Richmond, CA, USA) (Everaert *et al.*, 1995). All plasma treatments were done under 3.7 Torr gas pressure for 60 s and at a RF power of 50 W. Oxygen (99.5%), argon (99.996%), carbon dioxide (99.99%), and ammonia (99.98%) gases were obtained from Hoekloos Nederland B.V., Groningen, The Netherlands. As the hydrophobic recovery of the treated silicone rubber occurred within a few hours, plasma treatments were repeated every 24 h, while storing in ambient air in between each treatment. This cycle was repeated up to six times. After the sixth treatment, samples were stored for a period of 3 months in ambient air, Millipore grade water, or in liquid nitrogen (77K).

Prior to contact angle measurements or XPS, samples stored in liquid nitrogen were brought to ambient conditions by flushing the treated surface for 10 min with argon to prevent any ice condensation; samples stored in water were dried under reduced pressure for 10 min. Samples once used for surface characterization were not used again. All values given in this paper are the average of two separately prepared samples.

Contact angle measurements

Advancing and receding water contact angles were measured on each treated sample 10 min and 24 h after the samples had been brought to ambient conditions using the sessile drop technique (Everaert *et al.*, 1995). The advancing and receding angles were obtained by keeping the needle in the water droplet after positioning on the surface and by carefully moving the sample until the advancing angle

appeared to be maximal. Each reported value was obtained by averaging data for at least ten droplets, placed over different parts of the sample surface.

X-ray photoelectron spectroscopy

XPS was performed on each treated sample, 1 month after samples had been brought to ambient conditions, in order to avoid measuring on recovering samples, using an S-Probe spectrometer (Surface Science Instruments, Mountain View, CA, USA) as previously described (Everaert *et al.*, 1995). Binding energies were determined by setting the binding energy of the C_{1s} component due to carbon involved in siloxane (C-Si-O-Si) bonds at 284.5 eV (Beamson and Briggs, 1992). All Si_{2p} bands were fitted by fixing the distance between $Si_{2p(3/2)}$ and $Si_{2p(1/2)}$ at 0.7 eV and by imposing an intensity ratio of 2.0. Elemental surface compositions were expressed in atomic %, setting % C + % O + % Si + % N to 100%.

RESULTS

Water contact angles

Fi.s I and 2 compare the advancing and receding water contact angles, respectively, on silicone rubber treated six times in oxygen, argon, carbon dioxide, and ammonia plasmas and after a storage period of 3 months in ambient air, water, or in liquid nitrogen. Hydrophobic recovery, judged from the advancing water contact angles (Fig. 1), of oxygen and ammonia plasma-treated silicone rubber is almost completely suppressed during storage in water, or in liquid nitrogen, compared with storage in ambient air. However, within 24 h after returning the samples stored in liquid nitrogen to ambient air, hydrophobic recovery becomes comparable to the hydrophobic recovery occurring during storage in ambient air. After 24 h exposure to ambient air, samples treated by oxygen and ammonia plasmas after storage in water remain more hydrophilic than those stored in ambient air, or in liquid nitrogen. Also argon and carbon dioxide plasma-treated silicone rubber stored in liquid nitrogen remained considerably more hydrophilic compared to samples stored in ambient air, but in both cases the hydrophobic recovery occurred during subsequent exposure to ambient air, especially for carbon-dioxide treated silicone rubber.

The hydrophobic recovery of argon, carbon dioxide, and ammonia plasma-treated samples as judged from the receding water contact angles indicate trends similar to those when the recovery is judged from the advancing angles. Interestingly, however, hydrophobic recovery of the oxygen plasma-treated silicone rubber from receding water contact angles appears much smaller than from the advancing angles.

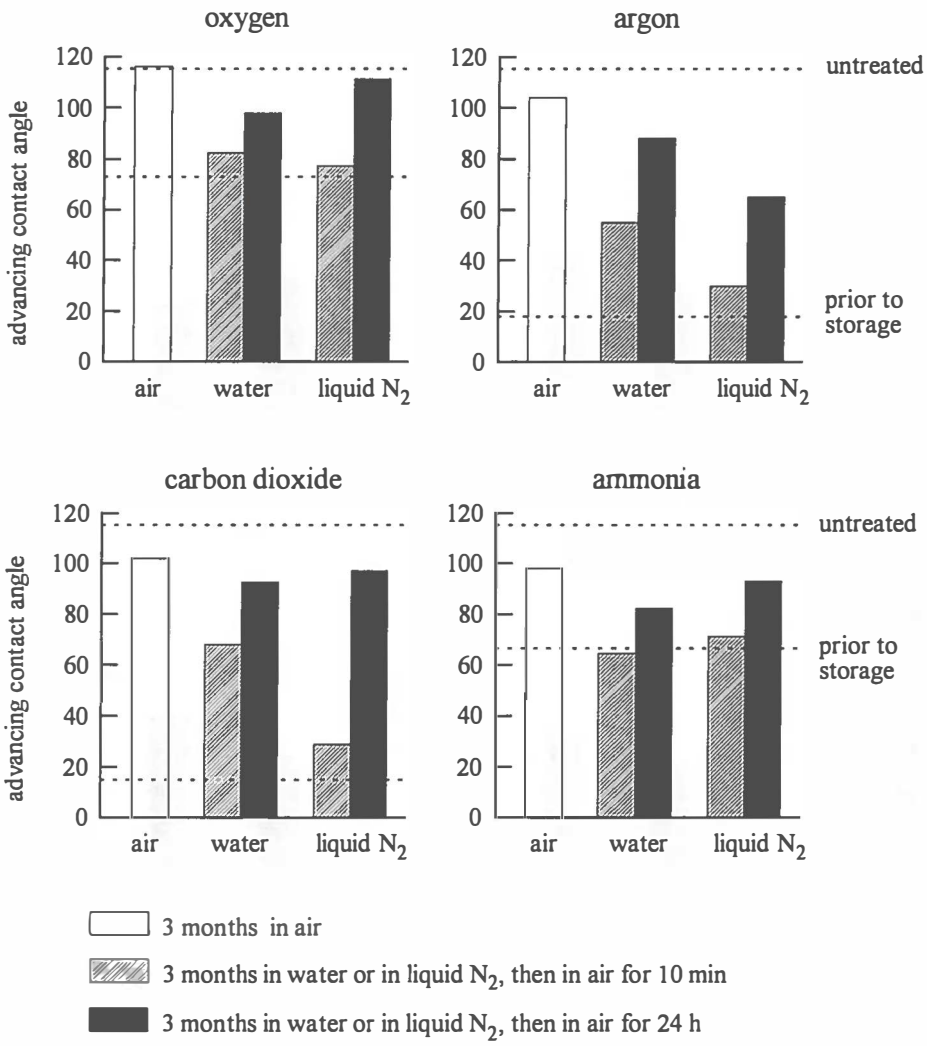


Figure 1. *Advancing water contact angles (degrees) on silicone rubber treated six times in various plasmas (50 W, 3.7 Torr, 60 s) after storage under various conditions. Samples stored 3 months in water or in liquid nitrogen were brought to ambient conditions prior to contact angle measurements. Measurements were done 10 min and 24 h after exposure to ambient air. The solid lines represent the advancing water contact angle for untreated silicone rubber (upper) and for silicone rubber immediately after the sixth plasma treatment i.e. prior to storage (lower). The standard deviation over ten measurements on one sample amounted to 3° on average, while the results for two separately prepared samples coincided within 7°.*

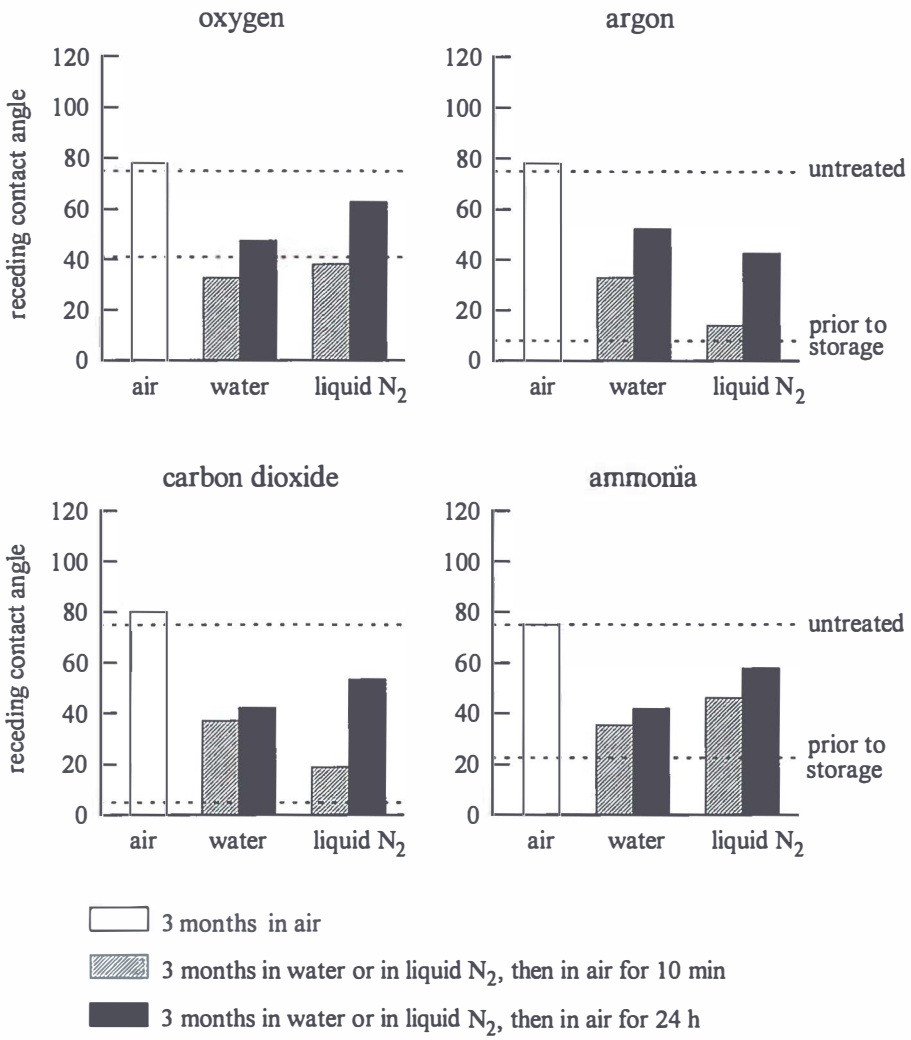


Figure 2. *Receding water contact angles (degrees) on silicone rubber treated six times in various plasmas (50 W, 3.7 Torr, 60 s) after storage under various conditions. Samples stored 3 months in water or in liquid nitrogen were brought to ambient conditions prior to contact angle measurements. Measurements were done 10 min and 24 h after exposure to ambient air. The solid lines represent the receding water contact angle for untreated silicone rubber (upper) and for silicone rubber immediately after the sixth plasma treatment i.e. prior to storage (lower). The standard deviation over ten measurements on one sample amounted to 3° on average, while the results for two separately prepared samples coincided within 5°.*

Elemental surface composition

The elemental surface composition of untreated silicone rubber as determined by the XPS measurements is 51.5% C, 25.4% O and 23.1% Si. Control experiments demonstrated that storage for 3 months in ambient air, water, or in liquid nitrogen did not affect this composition.

Figure 3 presents the elemental surface composition of plasma-treated silicone rubber in various gases, after a storage period of 3 months in ambient air, water, or in liquid nitrogen. Compared with samples stored in ambient air, the elemental surface composition observed after storage in water, or in liquid nitrogen, of argon, carbon dioxide and ammonia-treated silicone rubber revealed an increase in carbon and a decrease in the oxygen and silicon percentages; while oxygen plasma-treated surfaces yielded an increase in oxygen after storage at the expense of the carbon content. Nitrogen was not detected by the XPS except in the case of the ammonia-treated samples, for which the nitrogen content was measured to be 3, 0 and 2.5 for samples stored in ambient air, water, or in liquid nitrogen, respectively.

DISCUSSION

For many biomedical applications, the use of plasma-modified silicone rubber is limited by the fact that polymer surface dynamics causes partial or even complete disappearance of the surface modification effects over time. Although repeated plasma treatment already reduces the degree of hydrophobic recovery occurring (Everaert *et al.*, 1995), long term storage in ambient air still results in an appreciable hydrophobic recovery, as demonstrated in this study. This study also shows that the hydrophobic recovery is almost completely suppressed in liquid nitrogen and reduced in water, which may be of considerable importance for many applications of plasma-modified silicone rubber. For completeness it is noted that minor cracking occurs after repeated plasma treatments as previously demonstrated by SEM (Everaert *et al.*, 1995), but the extent to which this occurred was extremely small and hardly influenced the surface roughness as determined profilometrically.

The hydrophobic recovery is governed by an interplay of intrinsic materials properties such as the mobility of polymer chains, the presence of additives, and the degree of crosslinking, but also by environmental factors such as the hydrophilicity/hydrophobicity of the storage medium and temperature. Moreover, by rotational and translational motions of polymer chains or chain segments, freshly modified polymer surfaces adapt their surfaces to the environment, in order to minimize the interfacial free energy. Previously, van der Mei *et al.* (1991) observed that hydrophobic recovery of the oxygen plasma-treated polyethylene was absent during storage in liquid nitrogen. Morra *et al.* (1990), in a study on the aging of oxygen plasma-treated polydimethylsiloxane (PDMS) surfaces, reported faster hydrophobic recovery at elevated temperatures up to 393K than at room temperature,

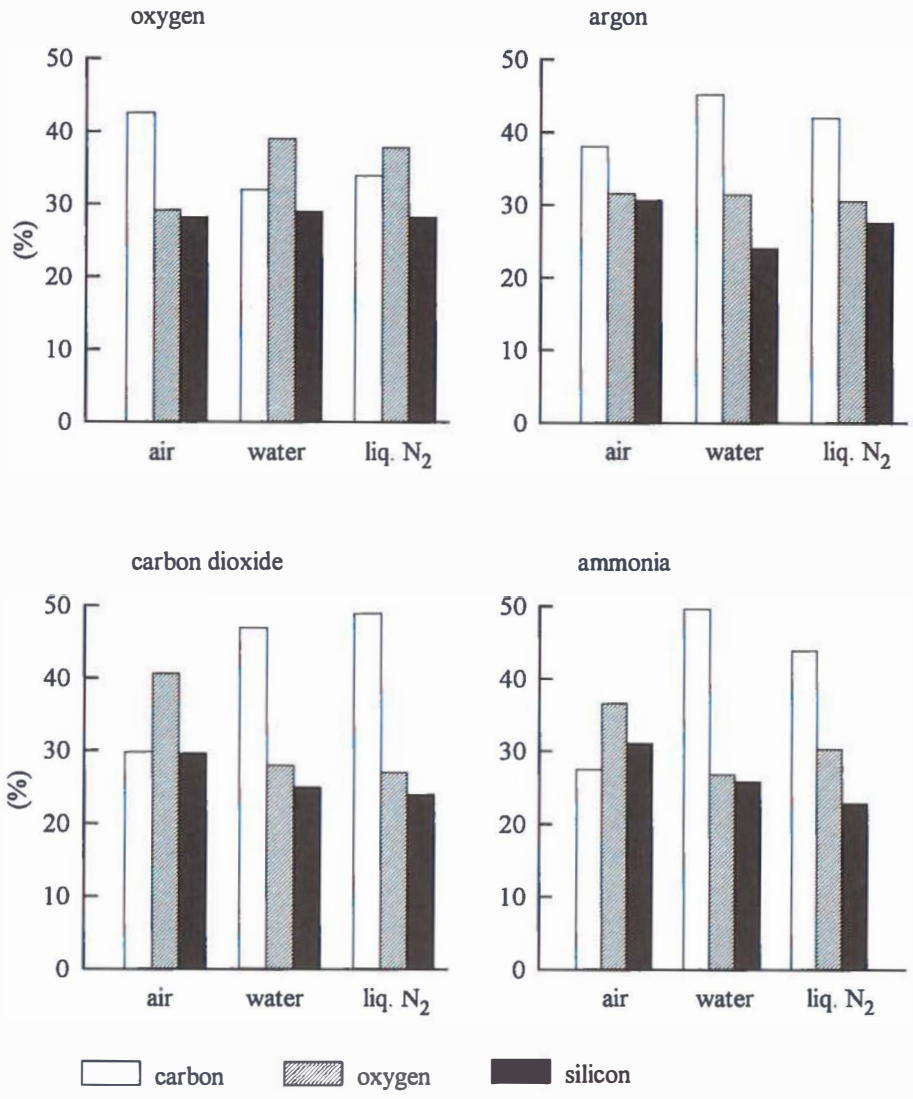


Figure 3. *Elemental surface compositions by XPS at an electron take-off angle of 55° to the normal of the sample of silicone rubber treated six times in various plasmas (50 W, 3.7 Torr, 60 s) after storage in ambient air, water or in liquid nitrogen. The standard deviation over three measurements on one sample amounted to 2% on average, while the results for two separately prepared samples coincided within 4%.*

while ruling out any thermal oxidation effects or adsorption of low-energy atmospheric contaminants. Lavielle and Schultz (1985), in a study on the interaction between water and acrylic acid-grafted polyethylene, reported that polar groups were attracted to the uppermost surface by its contact with water, demonstrating that polymer surfaces can indeed adapt their composition to the hydrophobicity/hydrophilicity of their environment.

It is known that polymers after plasma treatment may have a layer of water-soluble material on their surfaces, generally constituted of LMWOM (low-molecular-weight oxidized material) (Foerch *et al.*, 1993; Strobel *et al.*, 1989). Several studies have reported that ultrasonic treatment of plasma or corona-treated polymers in water may remove oxidized species, thereby increasing the hydrophobicity of treated polymers (Strobel *et al.*, 1989; Gerenser *et al.*, 1985). Also exposure to water without sonication of air plasma-treated polyethylene can result in a loss of oxygen as detected by the XPS (Foerch *et al.*, 1993). Therefore, it is most likely that, also in our study, water-soluble LMWOM of our plasma-treated surfaces is dissolved during storage in water.

Since the water contact angles of repeatedly oxygen and ammonia-treated silicone rubber hardly increase during storage in water (see Figs. 1 and 2), it is concluded that these plasmas do not yield any water-soluble LMWOM. Moreover, for oxygen plasma-treated surfaces stored in water, or in liquid nitrogen, an increase in oxygen percentage was systematically detected by the XPS, illustrating that ongoing oxidation may occur for these treated samples. However, as repeatedly argon and carbon dioxide plasma-treated silicone rubber shows a major increase in water contact angle after storage in water, it is suggested that these plasmas create water-soluble LMWOM on the silicone rubber.

CONCLUSION

This study shows that the hydrophobic recovery, judged from both advancing and receding water contact angles, of repeatedly plasma-treated silicone rubber is minimal during storage in water and is suppressed in liquid nitrogen, compared with storage in ambient air. However, during subsequent exposure to air, hydrophobic recovery is initiated again. Both XPS analyses and the water contact angles indicated that during storage of oxygen plasma-treated silicone rubber in water or in liquid nitrogen, ongoing oxidation may occur.

REFERENCES

Andersson L-O, Golander C-L and Persson S (1994), Ice adhesion to rubber materials. *J. Adhesion Sci. Technol.* **8**:117-132.

Beamson G and Briggs D (Eds) (1992), In: High Resolution XPS of Organic Polymers. The Scienta ESCA 300 Database, John Wiley & Sons, Chichester, 268-269.

Everaert EP, van der Mei HC, de Vries J and Busscher HJ (1995), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part I. Storage in air. *J. Adhesion Sci. Technol.* 9:1263-1278.

Foerch R, Kill G and Walzak MJ (1993), Plasma surface modification of polyethylene: short-term vs. long-term plasma treatment. *J. Adhesion Sci. Technol.* 7:1077-1089.

Morra M, Occhiello E and Garbassi F (1989), Contact angle hysteresis on oxygen plasma-treated polypropylene surfaces. *J. Colloid Interface Sci.* **132**:504-508.

Morra M, Occhiello E, Marola R, Garbassi F, Humphrey P and Johnson D (1990), On the aging of oxygen plasma-treated polydimethylsiloxane surfaces. *J. Colloid Interface Sci.* **137**:11-24.

Gaboury SR and Urban MW (1990), Spectroscopic evidence for Si-H formation during microwave plasma modification of poly(dimethylsiloxane) elastomer surfaces. *Polym. Comm.* **32**:390-392.

Garbassi F, Morra M, Occhiello E, Barino L and Scordamaglia R (1989), Dynamics of macromolecules: a challenge for surface analysis. *Surf. Interface Anal.* **14**:585-589.

Gerenser LJ, Elman JF, Masson MG and Pochan JM (1985), ESCA studies of corona-discharge-treated polyethylene surfaces by use of gas-phase derivatization. *Polymer* **26**:1162-1166.

Hollahan JR and Carlson GL (1970), Hydroxylation of polymethylsiloxane surfaces by oxidizing plasmas. *J. Appl. Polym. Sci.* **14**:2499-2508.

Lavielle L and Schultz J (1985), Surface properties of graft polyethylene in contact with water. *J. Colloid Interface Sci.* **106**:438-445.

Owen MJ, Gentle TM, Orbeck T and Williams DE (1988), Dynamic Wettability of Hydrophobic Polymers, In: *Polymer Surface Dynamics*, J.D. Andrade (Ed.), Plenum Press, New York, pp 101-110.

Owen MJ and Smith PJ (1994), Plasma treatment of polydimethylsiloxane. *J. Adhesion Sci. Technol.* **8**:1063-1075.

Strobel M, Dunatov C, Strobel JM, Lyons CS, Perron SJ and Morgen MC (1989), Low-molecular-weight materials on corona-treated polypropylene. *J. Adhesion Sci. Technol.* **3**:321-335.

Strobel M, Lyons CS and Mittal KL (Eds) (1994), In: *Plasma Surface Modification of Polymers: Relevance to Adhesion*, VSP, Zeist, The Netherlands.

Triolo PM and Andrade JD (1983), Surface modification and evaluation of some commonly used catheter materials. I. Surfaces properties. *J. Biomed. Mater. Res.* **17**:129-147.

Van der Mei HC, Stokroos I, Schakenraad JM and Busscher HJ (1991), Aging effects of repeatedly glow-discharged polyethylene: influence on contact angle, infrared absorption, elemental surface composition, and surface topography. *J. Adhesion Sci. Technol.* **5**:757-769.

A QUANTITATIVE MODEL FOR THE SURFACE RESTRUCTURING OF REPEATEDLY PLASMA TREATED SILICONE RUBBER

E.P. Everaert, R.C. Chatelier, H.C. van der Mei, and H.J. Busscher*

ABSTRACT Surface restructuring in ambient air of medical grade silicone rubber surfaces modified by repeated RF plasma treatments using various discharge gases including oxygen, argon, carbon dioxide and ammonia, was studied quantitatively. From advancing and receding water contact angle data, the fraction of the surface covered by mobile and immobile polar groups, and a characteristic time constant of the restructuring process were calculated. For argon plasma-treated surfaces, the fraction of immobile polar groups increased with repeated plasma treatments, but remained relatively constant for samples repeatedly treated by an ammonia plasma. The use of an oxygen plasma only yielded incorporation of mobile polar groups but not of immobile polar groups. The increase in the restructuring time constants of argon and ammonia plasma treated silicone rubber with the number of plasma treatments suggested enhanced crosslinking of the silicone rubber by these plasmas. In contrast, when an oxygen plasma was repeatedly used, the restructuring time constant decreased suggesting chain cleavage by an oxygen plasma. Tentatively, the carbon dioxide plasma treatment of silicone rubber may initially (up to 3-4 repeated treatments) yield chain cleavage, while the occurrence of crosslinking is indicated after more repetitions.

INTRODUCTION

Plasma treatments are well-known to improve the wettability of polymer surfaces when inherently high hydrophobicity limits certain applications (Strobel *et al.*, 1994; Liston *et al.*, 1993). However, restructuring of plasma-treated polymer surfaces, in response to their environment, often leads to a loss of the hydrophilicity created by the plasma (Liston *et al.*, 1993; Morra *et al.*, 1989, 1990; Garbassi *et al.* 1989; Owen *et al.*, 1988; van der Mei *et al.*, 1991; Everaert *et al.*, 1995, 1996; Chatelier *et al.*, 1995a, b; Owen and Smith, 1994). This so-called "hydrophobic recovery" is caused by the rotational and translational mobility of polymer chains and chain segments in the treated surface layer in order to minimize the interfacial free energy between the polymer and its environment, most frequently ambient air. Recently, we reported that the hydrophobic recovery of plasma treated silicone rubber could be delayed by storing the treated surfaces in a hydrophilic medium, such as water or at extremely low temperature, i.e. in liquid nitrogen (Everaert *et al.*, 1996).

*With permission of Plenum Press, Plasmas and Polymers, 2: 41-51 (1997).

Qualitatively, the restructuring of plasma treated polymers, also called "aging", has been described by means of water contact angle measurements (Liston *et al.*, 1993; Morra *et al.*, 1989, 1990; Garbassi *et al.* 1989; Owen *et al.*, 1988; van der Mei *et al.*, 1991; Everaert *et al.*, 1995, 1996; Chatelier *et al.*, 1995a, b), X-ray photoelectron spectroscopy (XPS) (Liston *et al.*, 1993; Morra *et al.*, 1989, 1990; Garbassi *et al.* 1989; Owen *et al.*, 1988; Van der Mei *et al.*, 1991; Everaert *et al.*, 1995, 1996; Chatelier *et al.*, 1995b; Owen and Smith, 1994) and by nuclear magnetic resonance (NMR) spectroscopy (Pfleiderer *et al.*, 1995). Quantitatively, however, surface restructuring of plasma treated polymers is insufficiently understood. Chatelier *et al.* (1995a, b) recently proposed a model to calculate the fraction of mobile and immobile polar groups on plasma treated polymer surfaces, and a characteristic time constant of the restructuring process, based on the measurement of advancing and receding water contact angles as a function of the aging time. Application of the model to ammonia vapor plasma treated fluoroethylene-propylene (FEP) surfaces (Chatelier, 1995b), demonstrated that the fraction of mobile polar groups increased with increasing plasma treatment time up to an optimal treatment time of 45 s, indicating that extended plasma exposure enhances chain cleavage reactions rather than crosslinking.

It is possible to increase the effects of plasma treatment by repeating the treatment after hydrophobic recovery. Using repeated oxygen plasma treatment, water contact angles on polyethylene, initially 90°, could be permanently reduced to 50° (van der Mei *et al.*, 1991). Moreover, repeated plasma treatment of silicone rubber, especially with an argon plasma, also appeared to be effective in increasing the degree of crosslinking, therewith minimizing hydrophobic recovery.

Whereas we previously described the hydrophobic recovery of repeatedly plasma treated silicone rubber in a qualitative sense by water contact angle measurements, X-ray photoelectron spectroscopy, profilometry, zeta potential measurements and by scanning electron microscopy (Everaert *et al.*, 1995), it is the aim of this study to quantitatively analyse the surface restructuring during hydrophobic recovery of repeatedly plasma treated silicone rubber using the model of Chatelier *et al.* (1995a) and our published water contact angle data (Everaert *et al.*, 1995)

EXPERIMENTAL

Materials

A Silastic® Medical Grade Silicone Rubber kit (Q7-4750, Dow Corning) was purchased and 1-mm-thick 50x76 mm plates were produced following the procedures suggested by the manufacturer. Briefly, equal proportions of "part A" and "part B" were thoroughly blended together and injected into a mold at room temperature through a 3 mm diameter opening with a force of 3000 kg. Subsequently, the silicone rubber was immediately cured at 200°C for 50 minutes. Finally, samples were cleaned by

sonication in a 5 % RBS 35 (Omnilabo international B.V., Breda, The Netherlands) detergent solution (5 min, 150 W) and thoroughly rinsed in Millipore® grade water and absolute ethanol (>96%).

Plasma treatment

The silicone rubber samples were repeatedly plasma treated in an inductively coupled (13.56 MHz RF) PLASMOD instrument (Tegal Corporation, Richmond, CA, USA). The PLASMOD is commercially available and equipped with a cylindrical quartz-made reaction chamber (8 cm inner diameter, 15 cm length). Pumping down was done with a Balzers rotary pump (320 l/min) using a liquid nitrogen cold trap. All plasma treatments were done under 3.7 Torr gas pressure, for 60 s and at a RF power of 50W. Oxygen (99.5 %), argon (99.996 %), carbon dioxide (99.99 %) and ammonia (99.98 %) gases were obtained from Hoekloos Nederland B.V., Groningen, The Netherlands.

In between repeated plasma treatments the samples were stored in ambient air (temperature 23-25°C, relative humidity 35-40°) in disposable Petri dishes. After the appropriate treatment number, samples were used immediately for water contact angle measurements as a function of the aging time up to 24 h. As the hydrophobic recovery of the treated silicone rubber occurred within a few hours, plasma treatments were repeated every 24 h. This cycle was repeated 6 times. Samples once used for contact angle measurements were not used again. All values given in this paper are the means of experiments on two separately prepared samples.

Contact angle measurements

Advancing and receding water contact angles were measured at room temperature with an image analyzing system, using the sessile drop technique. The advancing and receding angles were obtained by placing the needle in the water droplet (1-1.5 μl) and carefully moving the sample until the advancing angle appeared to be maximal. On each separately prepared sample surface, at least ten droplets were placed over different parts of the sample surface, yielding on average standard deviation of 2 and 3 degrees in advancing and receding contact angles, respectively.

Analysis of the water contact angle data

The restructuring of a plasma treated polymer surface, in response to its environment, leads to the partial, or sometimes complete disappearance of the surface modification effects over time. Chatelier *et al.* (1995a), recently developed a model for the time dependence of the theoretical equilibrium water contact angles, θ_E , during restructuring of plasma treated polymer surfaces. The model is based on defining two distinct populations of polar groups on plasma-treated polymer surfaces, attached onto

mobile and *immobile* chain segments. Assuming an exponential decay of the surface population of mobile polar groups ($f_{p,m}$), the total polar fraction (f_p) may be expressed as

$$f_p(t) = f_{p,im} + f_{p,m}\, e^{-t/\tau} \tag{1}$$

where τ is a characteristic time constant for surface restructuring and ($f_{p,im}$) is the fraction of the surface area covered by immobile polar groups. Further development of the model can be done either on the basis of the Cassie (1948) equation for water contact angles on heterogeneous surfaces or on the basis of the Israelachvili-Gee (1989) equation. According to Cassie, the equilibrium contact angle can be expressed as

$$\cos\theta_E = f_p \cos\theta_E^p + f_{np}\cos\theta_E^{np} \tag{2a}$$

while the Israelachvili and Gee approach leads to

$$(1 + \cos\theta_E)^2 = f_p\,(1 + \cos\theta_E^p)^2 + f_{np}(1 + \cos\theta_E^{np})^2 \tag{2b}$$

in which f_{np} is the fraction of nonpolar surface groups, and θ_E^p and θ_E^{np} are the theoretical equilibrium contact angle of a surface containing only polar or nonpolar regions, respectively.
Since $f_{np} = 1 - f_p$, insertion of equation (1) in the Cassie equation (2a) yields

$$\begin{aligned}\cos\theta_E(t) = {} & (f_{p,im} + f_{p,m}\, e^{-t/\tau})\cos\theta_E^p + \\ & (1 - f_{p,im} - f_{p,m}\, e^{-t/\tau})\cos\theta_E^{np}\end{aligned} \tag{3a}$$

while analogously, the Israelachvili-Gee equation (2b) can then be expressed as

$$\begin{aligned}(1 + \cos\theta_E(t))^2 = {} & (f_{p,im} + f_{p,m}\, e^{-t/\tau})(1 + \cos\theta_E^p)^2 + \\ & (1 - f_{p,im} - f_{p,m}\, e^{-t/\tau})(1 + \cos\theta_E^{np})^2\end{aligned} \tag{3b}$$

Application of equations (3a) or (3b) requires the measurement of the three theoretical equilibrium contact angles θ_E^p, θ_E^{np} and θ_E, which is impossible in principle (Neumann and Good, 1972). Therefore, it is assumed that $\theta_E^p = 0$ and that θ_E^{np} (99°) can be derived from the advancing (θ_A) and receding (θ_R) water contact angles on untreated silicone rubber according to

$$\cos\theta_E^{np} = \frac{\cos\theta_A + \cos\theta_R}{2} \tag{4}$$

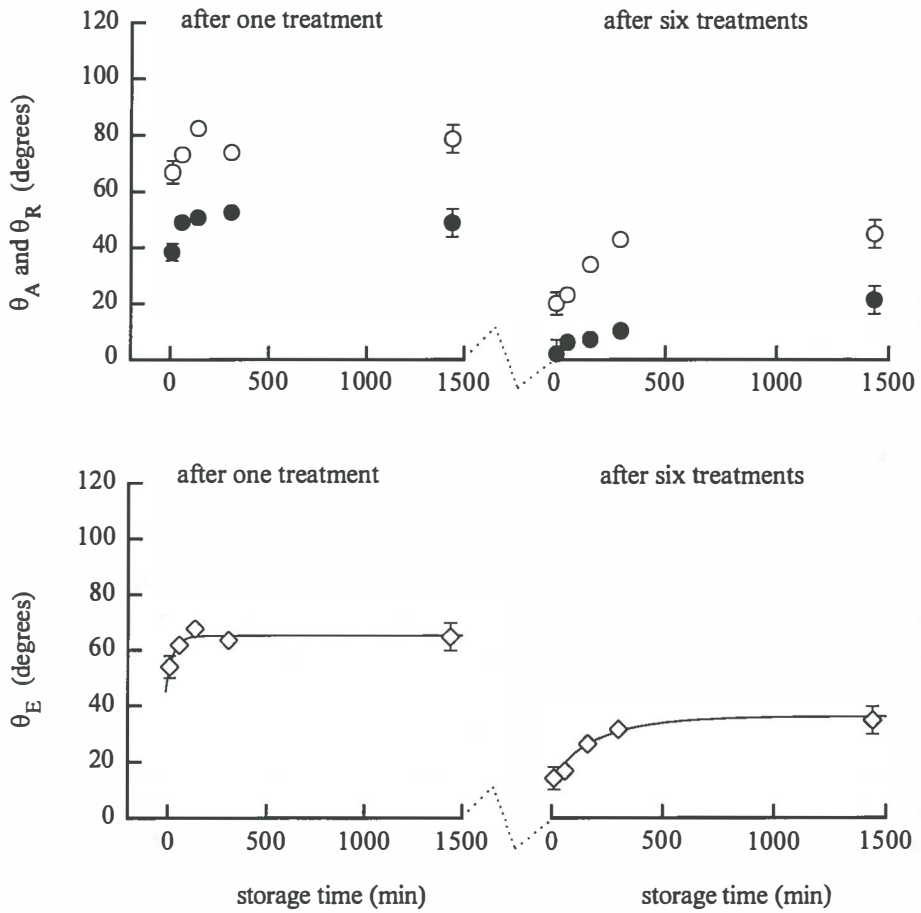


Figure 1. *Advancing (○) and receding (•) water contact angles of silicone rubber surfaces treated once (left) and six times (right) by an argon plasma as a function of storage time after each plasma treatment (top) and the corresponding values of equilibrium contact angle, θ_E, based on eq 5 (bottom), as a function of storage time. The best-fit curves based on the Cassie equation (3a) and the Israelachvili-Gee equation (3b) overlay one an other and are represented as a single solid line in the bottom graph.*

Analogously, from the time dependence of $\theta_A(t)$ and $\theta_R(t)$ of the plasma-treated silicone rubber during the restructuring process

$$\cos \theta_E(t) = \frac{\cos \theta_A(t) + \cos \theta_R(t)}{2} \quad (5)$$

Consequently, using measured contact angle data and equations (3a) or (3b), the fraction of mobile and immobile polar surface groups on a plasma treated polymer can be calculated, together with the characteristic time constant for the restructuring process by an iterative procedure, i.e. the Marquardt-Levenberg algorithm (Chatelier, 1995a).

RESULTS and DISCUSSION

From our published water contact angle data (Everaert *et al.*, 1995), $\theta_A(t)$ and $\theta_R(t)$ on repeatedly plasma treated silicone rubber surfaces, the parameters belonging to the restructuring process occurring in ambient air according to equation (3a) or (3b) have been calculated. These two equations (3a) and (3b) provided small (generally less than 3 %) differences in the restructuring parameters but yielded similar trends and conclusions. Fig. 1 reproduces a typical example of experimental values of θ_A and θ_R measured on silicone rubber surfaces repeatedly treated in an argon plasma as a function of storage time in ambient air, as well as the values of θ_E calculated via equation 5 and the best-fit curves based on the equations (3a) and (3b). The resultant best-fit parameters for all plasma treatments are described in the figs. 2 and 3.

Fig. 2 shows the fraction of the surface area covered by nonpolar regions (f_{np}), immobile polar groups ($f_{p,im}$) and by mobile polar groups ($f_{p,m}$), as a function of the number of plasma treatments. The degree to which repeated plasma treatment affected the polarity and the mobility of the silicone rubber surface clearly differed for the various gases used. A single carbon dioxide plasma treatment reduced the fraction of nonpolar surface groups to zero, while several argon plasma treatments were required to create a fully polar surface. Neither repeated oxygen nor repeated ammonia plasma treatments eventually yield fully polar surfaces, with $0.3 < f_{np} < 0.5$. All plasmas placed polar groups on the silicone rubber surface, but the fraction of immobile polar groups created by a repeated oxygen plasma treatment is virtually negligible, in line with full hydrophobic recovery observed (Everaert *et al.*, 1995). Repeated argon plasma treated silicone rubber had the most stable hydrophilic surface (Everaert *et al.*, 1995) and consequently the fraction of immobile polar groups created by an argon plasma is largest and increases with the number of treatments. Repeated carbon dioxide and ammonia plasma treatments yield varying fractions of mobile and immobile polar groups.

Fig. 3 shows the characteristic time constant of the restructuring process, τ, as a function of the number of plasma treatments. For silicone rubber surfaces modified by argon or ammonia

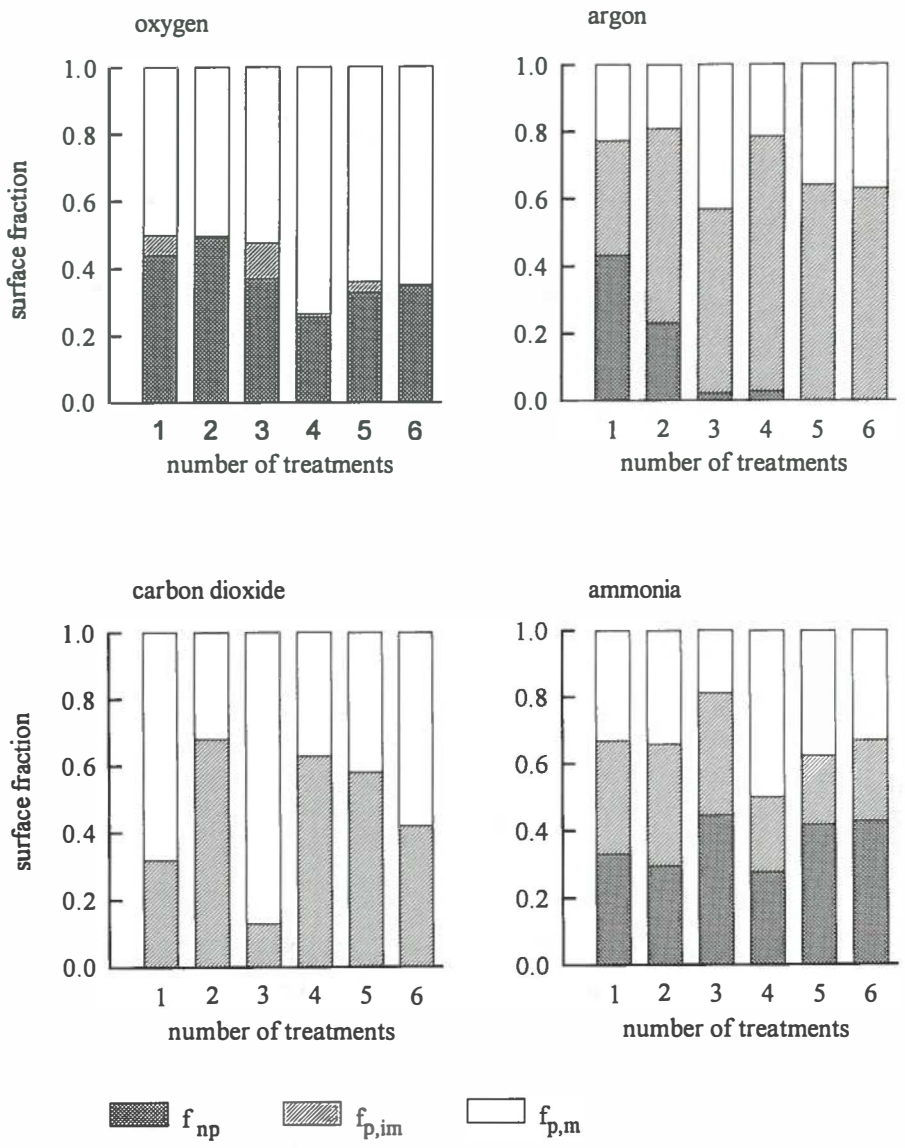


Figure 2. *Fraction of the surface covered by nonpolar f_{np}, immobile polar $f_{p,im}$ and by mobile polar groups $f_{p,m}$ on silicone rubber repeatedly treated in an oxygen, argon, carbon dioxide or ammonia plasma. All values were obtained by fitting the water contact angle data to the Israelachvili-Gee equation (3b).*

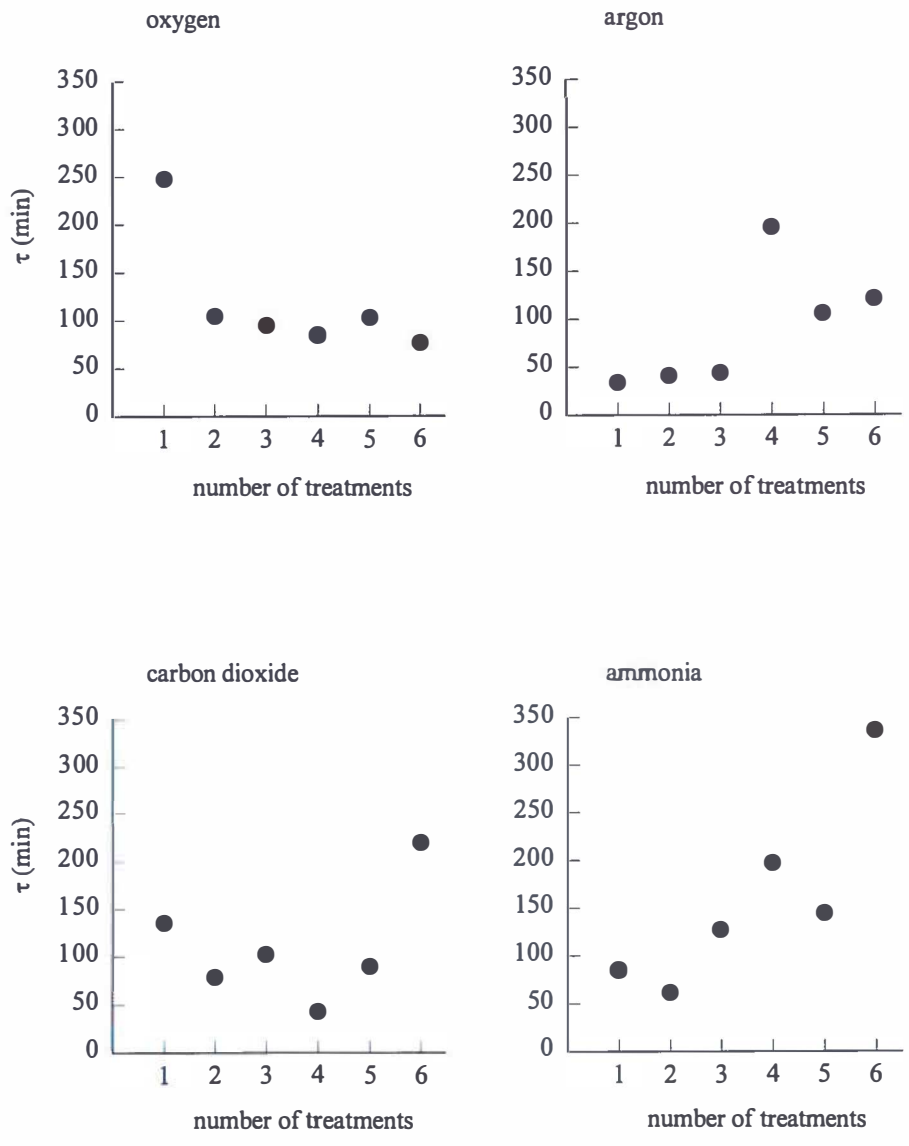


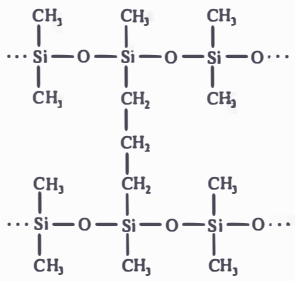
Figure 3. *Characteristic restructuring time constant, τ, for silicone rubber repeatedly treated in an oxygen, argon, carbon dioxide or ammonia plasma, obtained by fitting the water contact angle data to the Israelachvili-Gee equation (3b).*

plasma, restructuring time constants increase with repeated plasma treatments indicating that restructuring becomes slower upon repeating the plasma treatment. This suggests that, argon and ammonia repeated plasma treatments enhance crosslinking thereby reducing the mobility of the modified silicone rubber surface. For repeated oxygen plasma treatment an opposite relation between the restructuring time constant τ and the number of plasma treatments is observed. Thus repeated oxygen plasma treatment may stimulate cleavage of modified silicone rubber rather than crosslinking as do argon and ammonia plasmas. During fitting of the water contact angle data for carbon dioxide plasma treated silicone rubber it was difficult to obtain convergence in the Marquardt-Levenberg algorithm, and therefore interpretation of these results must be done with caution. Tentatively nevertheless, carbon dioxide plasma treatment of silicone rubber leads to chain cleavage, but after 3-4 repeated treatments crosslinking may occur.

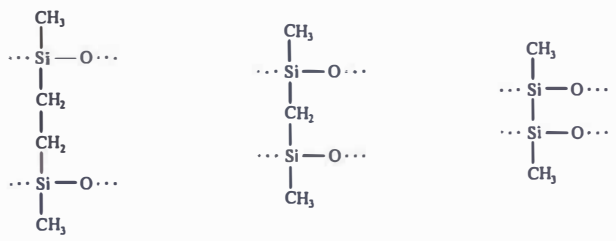
Urban and Stewart (1990) combined infrared spectroscopy (ATR-mode) and dynamic mechanical analysis to study the effects of single argon, carbon dioxide and ammonia plasma treatment (10 min, 50 W and 0.48 Torr) on industrial-grade silicone rubber. For ammonia plasma treated silicone rubber, higher glass transition temperatures and storage moduli were observed, which were interpreted to indicate increased crosslinking in accordance with the conclusions from the present study. Also in accordance with the present study, Urban and Stewart (1990) concluded that a single carbon dioxide plasma treatment causes chain cleavage. In contrast to our conclusions, however, no indications for crosslinking of argon plasma silicone rubber were found. Neither infrared spectroscopy nor dynamic mechanical analysis possess the extreme surface sensitivity of contact angle measurements and their results mainly reflect the bulk properties of the material under study. Therefore it is doubtful whether these techniques may be used to draw any conclusions about crosslinking in the surface regions of polymers due to plasma treatment.

It is interesting to speculate about the pathways by which silicone rubber is modified during and after repeated plasma treatment. Several types of reactive species are created in a plasma, including ions, radicals, free electrons and UV photons. Free radicals formed on a surface may react with each other, with species in the plasma, or with atmospheric oxygen when the chamber is vented after treatment. These reactions may result in the incorporation of polar groups into the polymer chains on the surface, chain scission or crosslinking. The medical-grade silicone rubber used here is composed of dimethyl and methylvinyl siloxane copolymers with reinforcing silica and platinum (1 ppm) as catalyst. During curing a propyl crosslink between two siloxane chains can form, as schematically shown in Fig. 4. Also shown in Fig. 4 are some suggestions on how radical recombination or (poly)condensation may yield cross-linked structures in silicone rubber (not all stable though). It is impossible to conclude on the basis of the present study which of the chemical structures represented in Fig. 4 are actually formed during plasma treatment of silicone rubber, and it is likely that more than

crosslinking in medical grade silicone rubber



crosslinking due to radical recombination



crosslinking due to (poly)condensation

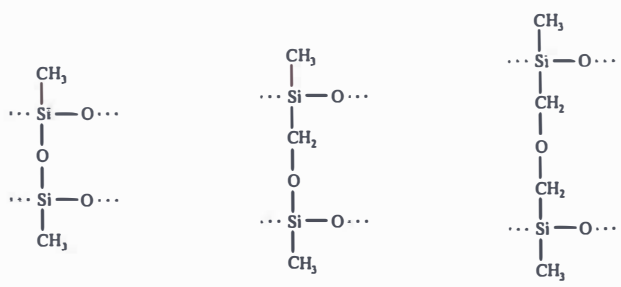


Figure 4. *Hypothetical chemical structures of crosslinking between siloxane chains in silicone rubber after plasma treatment, as may result either from radical recombination or (poly)condensation. Note that the third structure proposed under radical recombination may be photolytically unstable, while the second structure envisaged under (poly)condensation is possibly hydrolytically unstable.*

one of the structures suggested will be created. Previously (Everaert *et al.*, 1995), however, we concluded from the X-ray photoelectron spectroscopy that repeated plasma treatment of silicone rubber resulted in a new type of silicon with an Si_{2p} electron binding energy of 103.4 eV. The occurrence of this new type of silicon depended on the plasma used and correlated linearly with the O_{1s} electron binding energy component located at 533.2 eV (attributed to the oxygen included in new Si-O-Si or silica-like bonds). Thus it is likely that crosslinking in plasma treated silicone rubber will involve Si-O-Si bridges between the siloxane chains, as described in Fig. 4.

CONCLUSION

This study demonstrates that repeated treatments of silicone rubber with argon and ammonia plasmas lead to enhanced crosslinking in the surface layers of the elastomer. Oxygen plasma treatment, however, stimulates chain cleavage reactions.

REFERENCES

Cassie AB (1948), Contact angles. *Discuss. Faraday Soc.* **3**:11.

Chatelier RC, Xie X, Gengenbach TR, and Griesser HJ (1995a), Quantitative analysis of polymer surface restructuring. *Langmuir.* **11**:2576-2584.

Chatelier RC, Xie X, Gengenbach TR, and Griesser HJ (1995b), Effects of plasma modifications on surface restructuring. *Langmuir.* **11**:2585-2591.

Everaert EP, van der Mei HC, and Busscher H J (1996), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 2. A comparison of the hydrophobic recovery in air, water, or liquid nitrogen. *J. Adhesion Sci. Technol.* **10**:351-359.

Everaert EP, van der Mei HC, ve Vries J, and Busscher HJ (1995), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 1. Storage in air. *J. Adhesion Sci. Technol.* **9**:1263-1278.

Garbassi F, Morra M, Occhiello E, Barino L, and Scordamaglia R (1989), Dynamics of macromolecules:a challenge for surface analysis. *Surf. Interface Anal.* **14**:585-589.

Israelachvili JN, and Gee ML (1989), Contacts angles on chemically heterogeneous surfaces. *Langmuir.* **5**:288-289.

Liston EM, Martinu L, and Wertheimer MR (1993), Plasma surface modification of polymer for improved adhesion:a critical review. *J. Adhesion Sci. Technol.* **7**:1091-1127.

Morra M, Occhiello E, Marola R, Garbassi F, Humphrey P, and Johnson D (1990), On the aging of oxygen plasma-treated polydimethylsiloxane surfaces. *J. Colloid Interface Sci.* **137**:11-24.

Morra M, Occhiello E, and Garbassi F (1989), Contact angle hysteresis on oxygen plasma treatedpolypropylene surfaces. *J. Colloid Interface Sci.* **132**:504-508.

Neumann AW, and Good RJ (1972), Thermodynamics of contact angles. *J. Colloid Interface Sci.* **38**:341-358.

Owen MJ and Smith PJ (1994), Plasma treatment of polydimethylsiloxane. *J. Adhesion Sci. Technol.* **8**:1063-1075.

Owen MJ, Gentle TM, Orbeck T, and Williams DE (1988), Dynamic Wettability of hydrophobic polymers, In: *Polymer Surface Dynamics*, J.D. Andrade (Ed.), pp. 101-110. Plenum Press, New York.

Pfleiderer B, Xu P, Ackerman JL, and Garrido L (1995), Study of aging of silicone rubber biomaterials with NMR. *J. Biomed. Mater. Res.* **29**:1129-1140.

Strobel M, Lyons CS, and K.L. Mittal (Eds.) (1994), in: *Plasma Surface Modification of Polymers: Relevance to Adhesion*, VSP, Zeist, The Netherlands.

Urban MW, and Stewart MT (1990), DMA and ATR FT-IR studies of gas plasma modified silicone elastomer surfaces. *J. Appl. Polym. Sci.* **39**:265-283.

Van der Mei HC, Stokroos I, Schakenraad JM, and Busscher HJ (1991), Aging effects of repeatedly glow-discharged polyethylene: influence on contact angle, infrared absorption, elemental surface composition, and surface topography. *J. Adhesion Sci. Technol.* **5**:757-769.

IN VITRO AND *IN VIVO* MICROBIAL ADHESION AND GROWTH ON ARGON PLASMA TREATED SILICONE RUBBER VOICE PROSTHESES.

E.P.J.M. Everaert, H.F. Mahieu, A.M. Reitsma, B. Van de Belt-Gritter, H.C. van der Mei, G.J. Verkerke, F. Dijk and H.J. Busscher*

ABSTRACT Patients who underwent a total laryngectomy usually receive a silicone rubber voice prosthesis for voice rehabilitation. Unfortunately, biofilm formation on the esophageal side of voice prostheses limits their lifetime to 3-4 months on an average. In this paper, the effects of repeated argon plasma treatment of medical grade, hydrophobic silicone rubber on *in vitro* adhesion and growth of bacteria and yeasts isolated from voice prostheses, as well as *in vivo* biofilm formation will be presented. *In vitro* experiments demonstrated that initial microbial adhesion over a 4 h time span to plasma treated, hydrophilized, silicone rubber was generally less than on original, hydrophobic silicone rubber, both in the absence and presence of a salivary conditioning film on the biomaterial. Growth studies over a time period of 14 days at 37°C in a modified Robbins device showed that less *Candida* cells adhered on plasma treated, hydrophilized silicone rubber as compared to on original, hydrophobic silicone rubber. For the *in vivo* evaluation of biofilm formation on plasma treated silicone rubber voice prostheses, seven laryngectomized patients received a partly hydrophilized "Groningen button" voice prosthesis for a planned evaluation period of 4 weeks. After removal of the voice prostheses, the border between the hydrophilized and the original, hydrophobic side of the prostheses was clearly visible. However, biofilm formation was, unexpectedly, less on the original, hydrophobic sides, although the microbial compositions of the biofilms on both sides were not significantly different. Summarizing, this study demonstrates that *in vitro* microbial adhesion and growth on silicone rubber can be reduced by plasma treatment, but *in vivo* biofilm formation on silicone rubber voice prostheses is oppositely enhanced by hydrophilizing the silicone rubber surface. Nevertheless, from the results of this study the important conclusion can be drawn that *in vivo* biofilm formation on voice prostheses is controlled by the hydrophobicity of the biomaterials surface used.

INTRODUCTION

Total laryngectomy is a surgical treatment for extensive cancer of the larynx or hypopharynx. It entails surgical excision of the whole larynx including the vocal folds. Postoperatively the respiratory tract and upper digestive tract are totally separated, with a permanent tracheostoma in the neck as inlet and

*Submitted to The Journal of Materials Science: Materials in Medicine.

outlet of the respirawry tract. The anatomy of a patient after laryngectomy with a voice prosthesis is shown in Fig. 1. Acquisition of voice and intelligible speech following this procedure is considered the main determinant of the quality of life. During the last decade, surgical voice restoration procedures comprising tracheo-esophageal puncture techniques with insertion of a so called voice prosthesis have greatly improved successful voice acquisition following laryngectomy (Cooper *et al.*, 1989; Nijdam *et al.*, 1982; Mahieu *et al.*, 1987; Zijlstra *et al.*, 1991; Herrmann and Kley, 1981; Hilgers and Schouwenburg 1990; Hilgers and Balm, 1993; Traissac *et al.* 1987; Staffieri *et al.*, 1988; Algaba 1987; Singer and Blom, 1981). These voice prostheses consist of a valve situated in the surgically created tracheo-esophageal shunt which allows passage of air from the respiratory tract to the pharynx and the mouth, but prevents contents to pass from the digestive tract into the respiratory tract.

Speech is produced by closing the stoma with a finger and forcing air through the valve to the upper digestive tract, where remaining muscular structures at the esophageal entrance function as an alternative sound source. There are several types of voice prostheses available, such as the Groningen Button (Mahieu *et al.*, 1987; Zijlstra *et al.*, 1991), Herrmann-ESKA (Herrmann and Kley, 1981), Provox (Hilgers and Schouwenburg 1990; Hilgers and Balm, 1993), Traissac (Traissac *et al.* 1987), Staffieri (Staffieri and Staffieri, 1988), Algaba (Algaba, 1987) and Blom-Singer (Singer and Blom, 1981) voice prostheses. Most voice prostheses are made of silicone rubber because of its excellent mechanical and molding properties.

Voice prostheses are placed in a non-sterile, humid, nutrient-rich environment and therefore become quickly colonized by microorganisms (Mahieu *et al.*, 1986; Neu *et al.*, 1993; 1994a; 1994b; Busscher *et al.*, 1994; Izdebski *et al.*, 1987). Clinically, indwelling voice prostheses are replaced, on average, every four months (Van den Hoogen *et al.*, 1996) when, due to biofilm formation, patients complain about leakage of food and liquid or increased air flow resistance (Izdebski *et al.*, 1987). Analysis of the biofilms on voice prostheses removed from patients demonstrated that the initially colonizing yeast strains were mainly *Candida albicans* and that *Candida tropicalis* was probably the predominant yeast strain in mature biofilms on voice prostheses (Mahieu *et al.*, 1986; Neu *et al.*, 1993; 1994a). Bacterial strains identified were of oral origin and included *Streptococcus mitis*, *Streptococcus sobrinus* and *Streptococcus salivarius* or were commensals from the skin, such as *Staphylococcus epidermidis* and other staphylococcal isolates (Neu *et al.*, 1994b).

Prevention of colonization was partly achieved *in vivo* using antifungal amphotericin B lozenges (10 mg four times daily), which significantly prolonged the life time and therewith reduced the air flow resistance of the voice prostheses (Mahieu *et al.*, 1986). Prolonged administration of antifungal agents to patients is, however, uneconomical, indesirawe because of the danger to induce

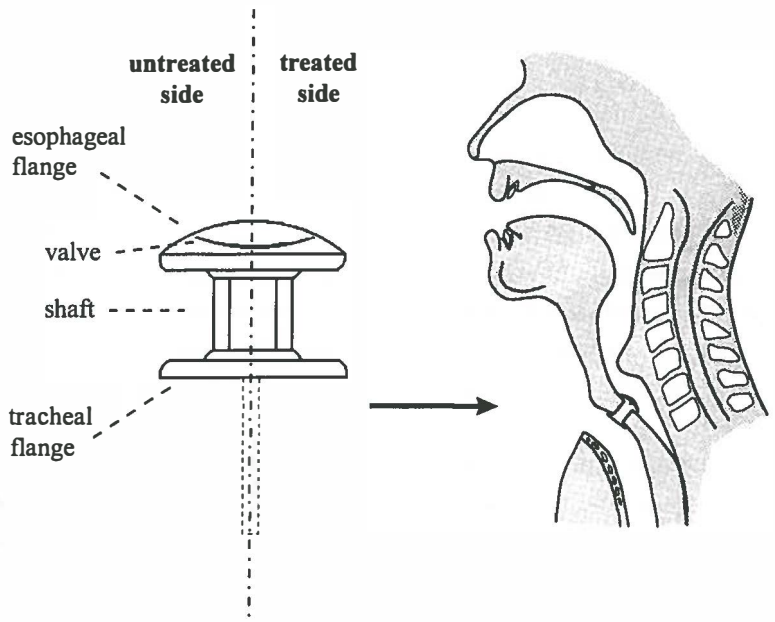


Figure 1. *Diagram of the "Groningen Button" voice prosthesis (left) and anatomy of the head-neck area after a total laryngectomy whith a "Groningen Button" voice prosthesis placed into the tracheo-esophageal shunt (right). Note that voice prostheses used for the evaluation of biofilm formation in vivo were only partly treated by argon plasma, as indicated in the figure.*

resistance, and the compliance of long term medication is low. Therefore it would be much more effective if deterioration of the prostheses by microorganisms could be prevented by changing the material or its surface characteristics.

The formation of a biofilm is usually considered to start with the adsorption of conditioning film components (Gristina, 1987), transport and adhesion of microorganisms, attachment and growth, possibly followed by ingrowth of selected organisms, most notably *C. tropicalis* and *C. albicans* in case of silicone rubber voice prostheses. Adsorption of conditioning film components and microbial adhesion to surfaces can be excellently studied *in vitro* using flow devices, such as a parallel plate flow chamber. Especially when combined with *in situ* observation methods and image analysis software, these systems have proved to be most versatile to study initial microbial adhesion phenomena (Busscher and van der Mei, 1995). Recently, also the biodeterioration of silicone rubber by ingrowing

yeasts has been simulated *in vitro* using a modified Robbins device by passing yeasts, adhering to silicone rubber, through a cycle of nutritional feast and famine (Busscher *et al.*, 1994).

Microbial adhesion to surfaces is an interplay of the hydrophobicity and charge properties of the interacting surfaces (Mozes *et al.*, 1988). Therefore, modification of the surface properties of silicone rubber by e.g. radio frequency plasma treatment, might reduce the adhesion and ingrowth of microorganisms to voice prostheses and therewith improve their life time. Plasma treatment is often a useful method to hydrophilize polymer surfaces, but effects of a single plasma treatment of silicone rubber are usually transient due to, amongst other reasons, the high mobility of the siloxane backbone. However, repeated plasma treatments of silicone rubber yields more permanent effects (Everaert *et al.*, 1995). Moreover, the hydrophilicity thus created onto silicone rubber surfaces can be preserved for several months by storing the plasma treated surfaces in a hydrophilic medium such as water (Everaert *et al.*, 1996).

The aim of this study is firstly to determine the influence of repeated argon plasma treatments of silicone rubber on initial microbial adhesion and yeast growth *in vitro*. To this end, adhesion of bacteria and yeasts to silicone rubber was studied in a parallel plate flow chamber, with and without an adsorbed salivary conditioning film. In addition, growth of yeasts adhering to silicone rubber was studied in a modified Robbins device. As a secondary aim, the clinical performance of argon plasma treated voice prostheses was evaluated.

MATERIALS AND METHODS

Strains and growth conditions

The bacterial and yeast strains used in this study were isolated from "Groningen Button" voice prostheses (Neu *et al.*, 1994b), removed from patients complaining either about leakage or blocking of the valve and included two bacterial strains *Streptococcus salivarius* GB 24/9, cultured in Todd Hewitt broth, *Staphylococcus epidermidis* GB 9/6, cultured in Tryptone Soya broth and the two yeasts strains (*Candida albicans* GB 1/2 and *Candida tropicalis* GB 9/9) cultured in Brain Heart Infusion broth. Culture media were purchased from Oxoid, Unipath LTD, Basingstoke, Hampshire, England. All microorganisms were inoculated from agar plates into a batch culture for 24 h at 37°C and ambient air, which was used to inoculate a second culture which was grown for 16 h under similar conditions.

The microorganisms were harvested by centrifugation (5 min at 4,000 *g* for bacterial and 10,000 *g* for the yeast strains), washed twice with Millipore® water and resuspended in adhesion buffer (50 mM KCl, 2 mM potassium phosphate and 1 mM $CaCl_2$, pH 6.8), bacteria to a concentration of 3×10^8 per ml and yeasts to a concentration of 3×10^6 per ml, as determined in a Bürker-Türk counting chamber.

Saliva coating

From healthy volunteers of both sexes, human whole saliva was collected into ice-chilled cups. Saliva was stimulated by the volunteers chewing Parafilm®. After the saliva was pooled and centrifuged at 10,000 *g* for 10 min at 4°C, phenylmethylsulfonylfluoride (0.2 M) was added to a final concentration of 1 mM as a protease inhibitor. The solution was again centrifuged, dialysed for 48 h at 4°C against Millipore® water and freeze dried for storage. A solution of 1.5 $mg.ml^{-1}$ of freeze dried stock in adhesion buffer will be denoted as (reconstituted human whole) saliva.

Silicone rubber and voice prostheses

Silastic® Medical Grade Silicone Rubber, a dimethyl and methylvinyl siloxane copolymer, (Q7-4750, Dow Corning) kit was purchased. Plates 0.5-mm-thick 50 x 76 mm (for flow chamber studies) or disks 1-mm-thick 6.3 mm diameter (for studies in the modified Robbins device) were produced following the procedures suggested by the manufacturer. Briefly, equal proportions of part A and B were thoroughly blended together and injected into a mold at room temperature. Subsequently, the silicone rubber was immediately cured at 200°C for 50 min. Finally, samples were cleaned in a 2% RBS 35 (Omnilabo International B.V., Breda, The Netherlands) detergent solution under simultaneous sonication (5 min, 150 W) and thoroughly rinsed in Millipore® grade water and absolute ethanol (>96%).

The "Groningen Button Ultra Low Resistance" voice prostheses were obtained from Medin Instruments and Supplies (Groningen, The Netherlands) and plasma treated as described below.

Repeated plasma treatment of silicone rubber

The silicone rubber plates (for use in the flow chamber), disks (for use in the modified Robbins device) and voice prostheses were repeatedly (six times with 24 h time interval in between) glow discharge treated in a DC modified Edwards sputter coater S150B with a cylindrical reaction chamber (inner diameter 15 cm, height 11 cm, electrode diameter 10 cm with a separation distance of 4.5 cm). All plasma treatments were done under 0.2 mbar argon pressure, at a power of 5 W for 5 min. Argon gas (99.996%) was obtained from Hoekloos Nederland B.V., Groningen, The Netherlands. Samples were stored in ambient air in between plasma treatment. After the last argon plasma treatment, treated samples were used immediately for parallel plate flow chamber or modified Robbins device experiments, while treated voice prostheses were stored in sterile water prior to placement in a patient. Treated voice prostheses were never stored longer than 3 months.

The silicone rubber disks for use in the modified Robbins device as well as the "Groningen

button" voice prostheses (see Fig. 1) were partly exposed to the argon plasma for only *half* of their surfaces by placing the samples in a plaster cast (Dental superhart gypsum, New Fujirock, GC Corporation, Tokyo, Japan), during treatments (Everaert *et al.*, 1997) Therewith, the growth experiments in the Robbins device as well as the clinical evaluation of the surface modification *in vivo* could be done on one disk or voice prosthesis, respectively.

Water contact angles were measured on each half of these samples, to ensure that the silicone rubber hydrophobicity had been maintained and that the plasma had created a hydrophilic surface. Typically, the advancing water contact angles on the hydrophobic side of a silicone rubber disk or voice prosthesis were 115 degrees, while the hydrophilized sides had advancing water contact angles of 15 degrees.

The parallel plate flow chamber and image analysis

The flow chamber and image analysis system have been previously described (Busscher and Van der Mei, 1995). Images were taken from the bottom plate of the parallel plate flow chamber which consisted of a silicone rubber or plasma treated silicone rubber sample affixed to a thicker (1.5 mm) perspex plate. The top plate of the chamber was made of glass.

Deposition was observed with a CCD-MXRi camera (High Technology, Eindhoven, The Netherlands) mounted on a phase contrast microscope (Olympus BH-2) equipped with a 40 x ultra long working distance objective (Olympus ULWD-CD Plan 40 PL) for experiments with bacteria and with a 10 x objective for experiments with yeasts. The camera was coupled to an image analyzer (TEA, Difa, Breda, The Netherlands). Each live image (512 x 512 pixels with 8 bits resolution), obtained after summation of 8 consecutive images (time interval 1 sec) in order to enhance the signal-to-noise ratio and to eliminate moving microorganisms from the analysis. Subsequently, adhering microorganisms were discriminated from the background by a single grey-value threshold yielding a binary black and white image and the number of adhering microorganisms was counted. An image covers a surface area of 0.017 mm^2 at the magnification used for bacterial experiments and 0.3 mm^2 at the magnification employed in the experiments with yeasts.

Prior to each experiment, all tubes and the flow chamber were filled with adhesion buffer, while care was taken to remove air bubbles from the system. Flasks, containing microbial suspension, buffer and saliva when appropriate, were positioned at the same height with respect to the chamber to ensure that immediately after the flows were started, all fluids would circulate through the chamber at the desired shear rate of 10 s^{-1} (0.025 $ml.s^{-1}$), which yields a laminar flow (Reynolds number 0.6).

When a salivary conditioning film was required, flow was switched first to saliva for 1.5 h, followed by a flow of buffer during 1 h to remove all remnants of saliva from the tubes and chamber.

The microbial suspension was circulated through the system for 4 h and images were obtained at the highest possible rate. The initial increase in the number of adhering microorganisms with time was expressed in a so-called initial deposition rate j_0 [$cm^{-2}.s^{-1}$], i.e. the number of microorganisms adhering per unit time and area. The number of microorganisms adhering after 4 h, n_{4h} was taken as an estimate of microbial adhesion in a more advanced state of the process. Finally, while focus was maintained on the same spot of the substratum, the number of adhering microorganisms in this field of view was compared with the number of microorganisms that remained adhering after passing an air-bubble through the chamber to obtain an indication of the adhesive forces (Pitt *et al.*, 1993). All values given in this paper are the means of experiments on at least two separately prepared samples.

Modified Robbins device

A modified Robbins device (Costerton *et al.*, 1986), in which 10 silicone rubber disks could be inserted simultaneously has been used. Silicone rubber disks used in the Robbins device were exposed for only half of their surface to the plasma treatment. First, the device was inoculated with an overnight culture of the appropriate yeast strain, either *C. albicans* GB 1/2 or *C. tropicalis* GB 9/9. The device was then perfused for 7 days with defined growth medium (glucose 7.5 $g.l^{-1}$, $(NH_4)_2SO_4$ 3.5 $g.l^{-1}$, L-asparagine 1.5 $g.l^{-1}$, L-histidine 10 $mg.l^{-1}$, DL-methionine 20 $mg.l^{-1}$, DL-tryptophane 20 $mg.l^{-1}$, KH_2PO_4 1 $g.l^{-1}$, $MgSO_4.7H_2O$ 500 $mg.l^{-1}$, NaCl 500 $mg.l^{-1}$, $CaCl_2.2H_2O$ 500 $mg.l^{-1}$, yeast extract 100 $mg.l^{-1}$, H_3BO_3 500 $\mu g.l^{-1}$, $ZnSO_4.7H_2O$ 400 $\mu g.l^{-1}$, $Fe(III)Cl_3$ 120 $\mu g.l^{-1}$, $Na_2MoO_4.2H_2O$ 200 $\mu g.l^{-1}$, KI 100 $\mu g.l^{-1}$, $CuSO_4.5H_2O$ 40 $\mu g.l^{-1}$) and subsequently, also for 7 days, with phosphate buffered saline (10 mM potassium phosphate and 150 mM NaCl, pH 7.0), in order to mimic the varying availability of nutrients occurring *in vivo*. The disks were removed after 14 days for scanning electron microscopy (SEM). The temperature of the device was maintained at 35-37°C during all experiments.

For electron microscopy, the disks were first flushed with 6.8% sucrose and 0.2 % ruthenium red in 0.1 M cacodylate buffer (pH 7.4). After fixation in 2% glutar-di-aldehyde and 0.1 M cacodylate buffer, for 2-24 h at 4°C, disks were flushed a second time. Post-fixation was carried out in 1% OsO_4 and 0.2% ruthenium red in 0.1 M cacodylate buffer by gently shaking for 3 h at room temperature. This procedure removed most of the thick biofilm present on the disks, leaving only those cells that were in direct contact with the silicone rubber and that potentially showed ingrowth.

Dehydratation involved sucrose and 0.1 M cacodylate buffer: 20 min; bidistilled water: 3 x 10 min; ethanol series, 30, 50 and 70%: each 20 min; ethanol 100%: 4 x 30 min. After critical-point drying with CO_2 for 4 h, the specimens were mounted on SEM stubs and sputter-coated with gold and palladium (15 nm).

Clinical evaluation of plasma treated voice prostheses

Seven laryngectomized patients with at least 6 months experience wearing a voice prosthesis, received a partly plasma treated Groningen Button voice prosthesis for a planned evaluation period of approximately four weeks. After removal from the patients, biofilm formation on the modified and unmodified side of the prosthesis were compared by visual and scanning electron microscopical evaluation, the latter as described for the sample disks in the Robbins device experiments. Visual evaluation included a planimetric evaluation of the percentage area of the esophageal flange covered by biofilm.

Furthermore, microbial compositions of the biofilms on modified and unmodified sides of the esophageal valves of the prostheses were compared by plating on Brain Heart Infusion and blood agar plates at 37°C under aerobic conditions (Neu *et al.*, 1994a). To this end, microorganisms were separately isolated from both the plasma treated and untreated side of the prostheses, transfered to 4.5 ml of reduced transport fluid (RTF) and sonicated for 20 s. Subsequently, the microbial suspension was diluted up to six times in RTF and from each dilution microorganisms were grown on Brain Heart Infusion and blood agar plates at 37°C in ambient air. Isolated bacterial colonies were first examined by light microscopy and Gram-staining. Then, the bacterial strains were identified by the Biolog system and the yeast strains by API ID 32C test system (BioMérieux).

RESULTS

In vitro flow chamber experiments

Fig. 2 presents an example of the adhesion kinetics for a microbial strain to argon plasma treated, hydrophilized and original, hydrophobic silicone rubber. Quantitative characteristics of these plots, including the initial deposition rate j_0, the adhesion in a stationary end point n_{4h} and the percentage of adhering microorganisms detached after the passage of an air-bubble through the flow chamber are shown in Figs. 3 and 4 for the bacterial and yeasts strains, respectively. As can be seen from Figs. 3 and 4, initial deposition rates to plasma treated, hydrophilized silicone rubber were generally lower than to hydrophobic silicone rubber both in the absence and presence of a salivary conditioning film. Similarly, also the numbers of adhering microorganisms after 4 h were lower for plasma treated, hydrophilized than for original, hydrophobic silicone rubber surfaces. In addition, some strains were more readily detached by the passage of an air-bubble from plasma treated, hydrophilized silicone rubber.

Although the above summarizing statements are not all equally valid for all strains tested here, the *in vitro* flow chamber studies appear to point out that argon plasma treatment could be a promising method to discourage biofilm formation on silicone rubber voice prostheses.

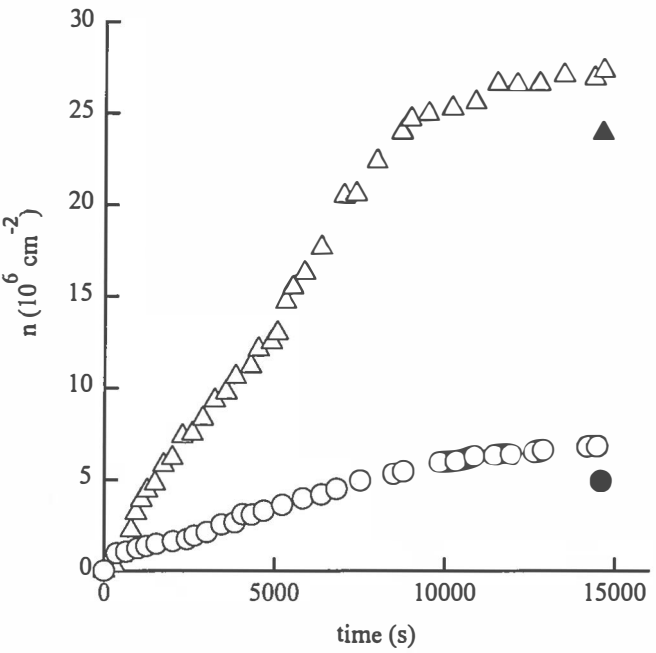


Figure 2. *Adhesion kinetics of Staph. epidermidis GB 9/6 to repeatedly argon plasma treated, hydrophilized (○) and original, hydrophobic silicone rubber (△). The filled symbols denote the number of adhering bacteria after passing an air-bubble through the flow chamber.*

In vitro Robbins device experiments

All disks removed from the Robbins device were covered by a thick biofilm, as could be seen with the naked eye, but during preparation for electron microscopy most of the biofilm detached, leaving only the microorganisms in direct contact with the silicone rubber surface.

Fig. 5 shows scanning electron micrographs of argon plasma treated, hydrophilized and original, hydrophobic sides of the silicone rubber disks after growth of *C. tropicalis* GB 9/9 and *C. albicans* GB 1/2, respectively, under dynamic nutrient conditions at 37°C. In line with the flow chamber studies, involving only adhesion and not growth, less *Candida* cells were found adhering on the hydrophilized side as compared to the original hydrophobic silicone rubber. No ingrowth of the adhering yeasts was observed.

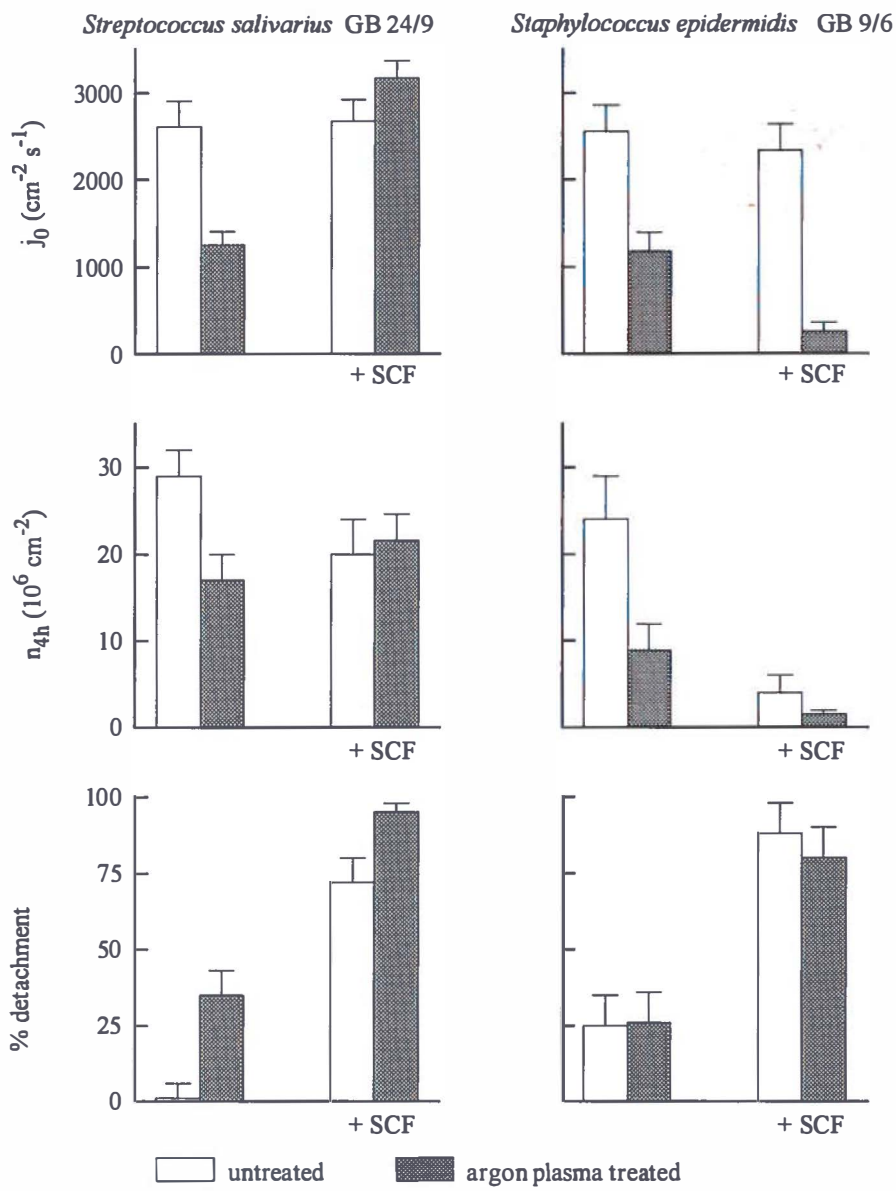


Figure 3. *Initial deposition rates j_0, numbers of adhering bacteria after 4 hours n_{4h}, and percentages of bacteria detached after passing an air-bubble through the flow chamber to argon plasma treated hydrophilized and original, hydrophobic silicone rubber in the absence and presence of a salivary conditioning film (SCF), for two bacterial strains; Strept. salivarius GB 24/9 (left hand panel) and Staph. epidermidis GB 9/6 (right hand panel).*

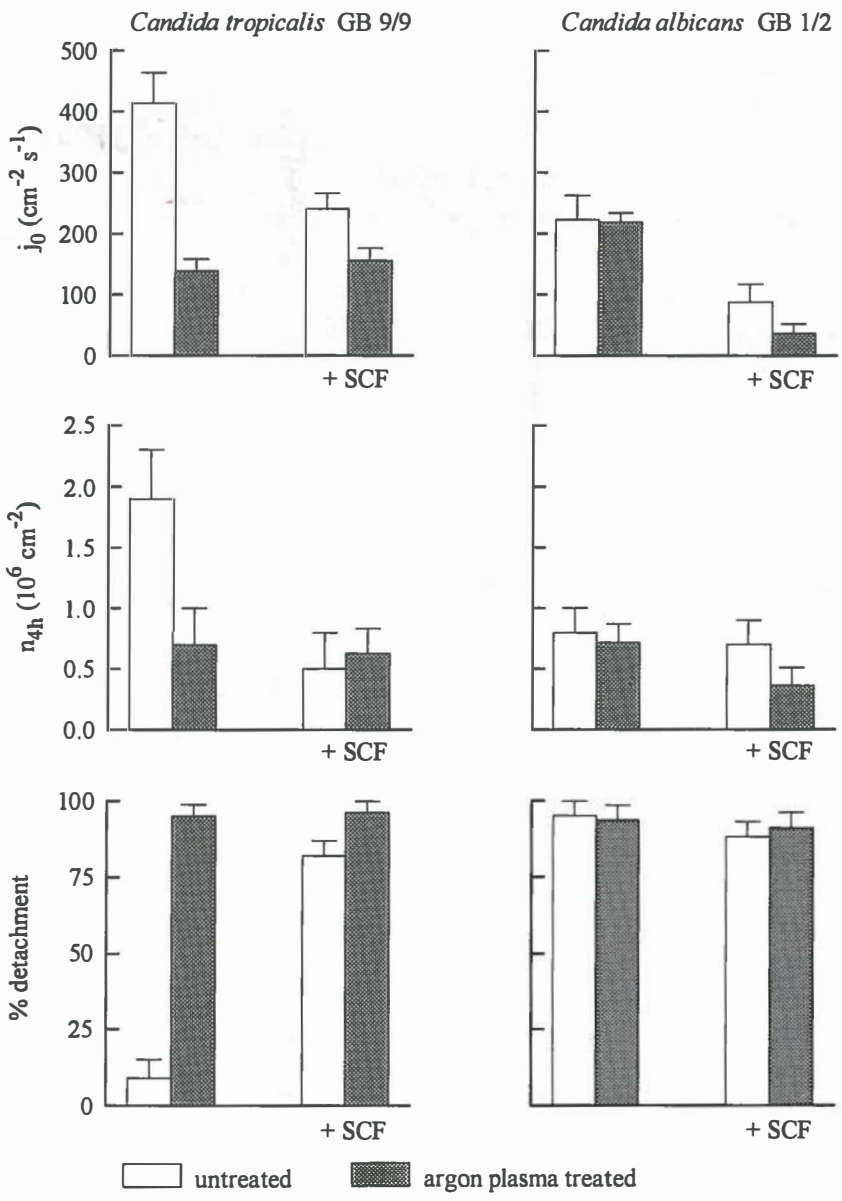


Figure 4. *Initial deposition rates j_0, numbers of adhering yeasts after 4 hours n_{4h}, and percentages of yeasts detached after passing an air-bubble through the flow chamber to argon plasma treated, hydrophilized and original, hydrophobic silicone rubber in the absence and presence of a salivary conditioning film (SCF), for two yeast strains; C. tropicalis GB 9/9 (left hand panel) and C. albicans GB 1/2 (right hand panel).*

Evaluation of in vivo biofilm formation on voice prostheses

In Fig. 6, it can be seen that the border between the plasma treated, hydrophilized and the original, hydrophobic side of the esophageal flange of the voice prostheses removed from laryngectomized patients is clearly visible from differential biofilm formation, which is significantly less on the original, hydrophobic side (Fig. 6a). Quantitative support for this statement can be found in Table I, summarizing the number of colony forming units (CFU) and planimetrically scored biofilm formation on both sides of the voice prostheses as removed from the patients. In all patients, hydrophilizing the silicone rubber enhanced biofilm formation. Ingrowth of adhering bacteria and yeasts strains was also seen, especially on the hydrophilized side, as shown in Fig. 6b, yielding crater-like defects surrounded by circular deformations (see Fig. 6c).

Table I. The number of colony forming units per unit area (CFU) and planimetric biofilm scores on partly hydrophilized "Groningen Button" silicone rubber voice prostheses removed from laryngectomized patients, after a planned evaluation period of four weeks.

Patient	original, hydrophobic side		hydrophilized side	
	planimetric biofilm score (%)	CFU ($10^6.cm^{-2}$)	planimetric biofilm score (%)	CFU ($10^6.cm^{-2}$)
A	30	0.2	90	8.6
B	10	3.0	100	56.2
C	20	0.1	30	38.7
D	10	3.3	70	41.1
E	20	-	40	-
F	5	0.2	30	1.6
G	30	3.9	90	117

Table II compares the microbial compositions of the biofilms on each side of the esophageal flange of prostheses removed from patients. Five different *Candida* species and 16 different bacterial strains were isolated. Among the *Candida* species isolated, no *C. tropicalis* strains were found, while the bacterial strains identified included commonly isolated strains and species like lactobacilli, staphylococci and streptococci. However, in general the plasma treated, hydrophilized side of the valves did not attract a significant number of different microbial strains and species as compared to the original, hydrophobic side.

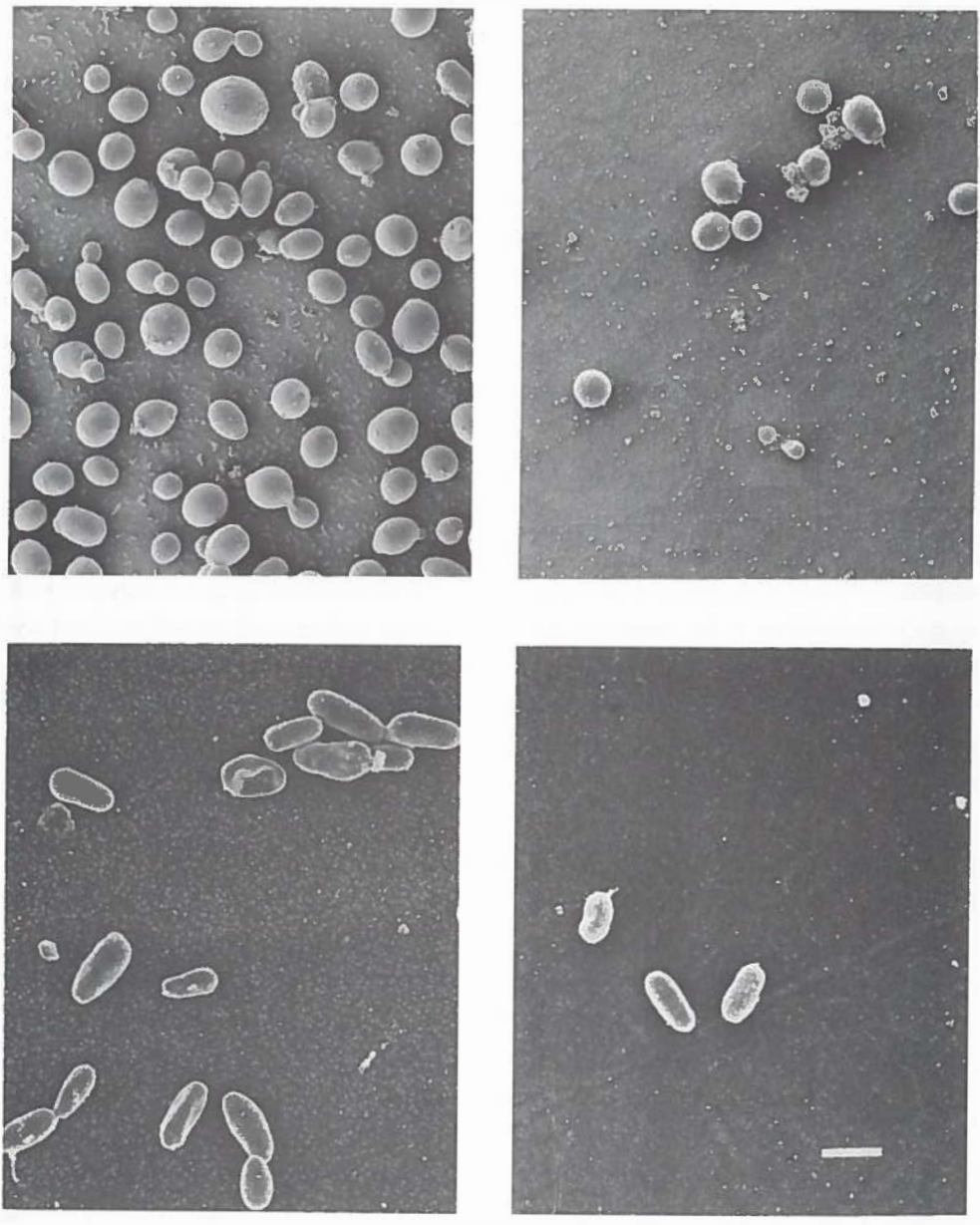


Figure 5. *Scanning electron micrographs of argon plasma treated, hydrophilized (right) and original, hydrophobic (left) silicone rubber, after growth of C. albicans GB 1/2 (top) or C. tropicalis GB 9/9 (bottom) in a modified Robbins device under dynamic nutrient conditions. The bar denotes 5 μm.*

DISCUSSION

In this paper we describe the *in vitro* and *in vivo* evaluation of the fouling properties of argon plasma treated, hydrophilized silicone rubber voice prostheses in laryngectomized patients. The evaluation therewith includes all steps in the development of a biofilm, ranging from adsorption of conditioning film components and initial adhesion of yeasts and bacteria, growth, possibly followed by ingrowth, as well as long term *in vivo* biofilm formation. Most interestingly, *in vitro* experiments involving two bacterial and two yeast strains isolated from voice prostheses indicated that hydrophilized silicone rubber might perform better *in vivo* than original, hydrophobic silicone rubber. Although *in vivo* experiments proved oppositely, evaluation of biofilm formation on partly hydrophilized voice prostheses most decisively showed that biofilm formation in the oro-pharyngeal region is governed by the hydrophobicity of the surfaces exposed.

Also *in vitro* studies on oral streptococcal adhesion to materials with and without a salivary conditioning film and comparisons with *in vivo* evaluation of dental plaque formation revealed such discrepancies (Pratt-Terpstra *et al.*, 1989; Quirynen *et al.*, 1989; 1991). In these studies, oral streptococcal adhesion to surfaces *in vitro* in the absence of a salivary conditioning film were governed by the hydrophobicity of the substratum surfaces, but when surfaces were covered by a salivary conditioning film, all surfaces attracted more or less similar numbers of adhering streptococci (Pratt-Terpstra *et al.* 1989). Dental plaque formation over a nine days time period on these materials when glued on the front incisors of human volunteers, however, showed far less plaque on hydrophobic than on hydrophilic materials (Quirynen *et al.*, 1989).

There are several reasons why *in vitro* and *in vivo* evaluation of the fouling properties of biomaterials surfaces might give contradictory results:

- The number of strains and species occurring *in vivo* and their variability in cell surface properties is much larger than can be evaluated *in vitro*.
- Co-adhesion phenomena between bacteria, yeasts as well as between yeasts and bacteria occur *in vivo* (Kolenbrander, 1988; Jenkinson *et al.*, 1990; Holmes *et al.*, 1995) but make *in vitro* evaluation even more difficult.
- The conditions in the oro-pharyngeal cavity are highly dynamic with regard to nutrient availability, temperature, humidity and shear.

The existence of periods with low and high shear, like during swallowing, eating and drinking puts special emphasis on the initially adhering microorganisms as a link between the biomaterial and the biofilm as a whole (Busscher *et al.*, 1995). It has been suggested that during periods of low shear, microorganisms adhere to the outer layer of the conditioning film, which may be relatively similar on different biomaterials, explaining why the microbial compositions on hydrophilized and

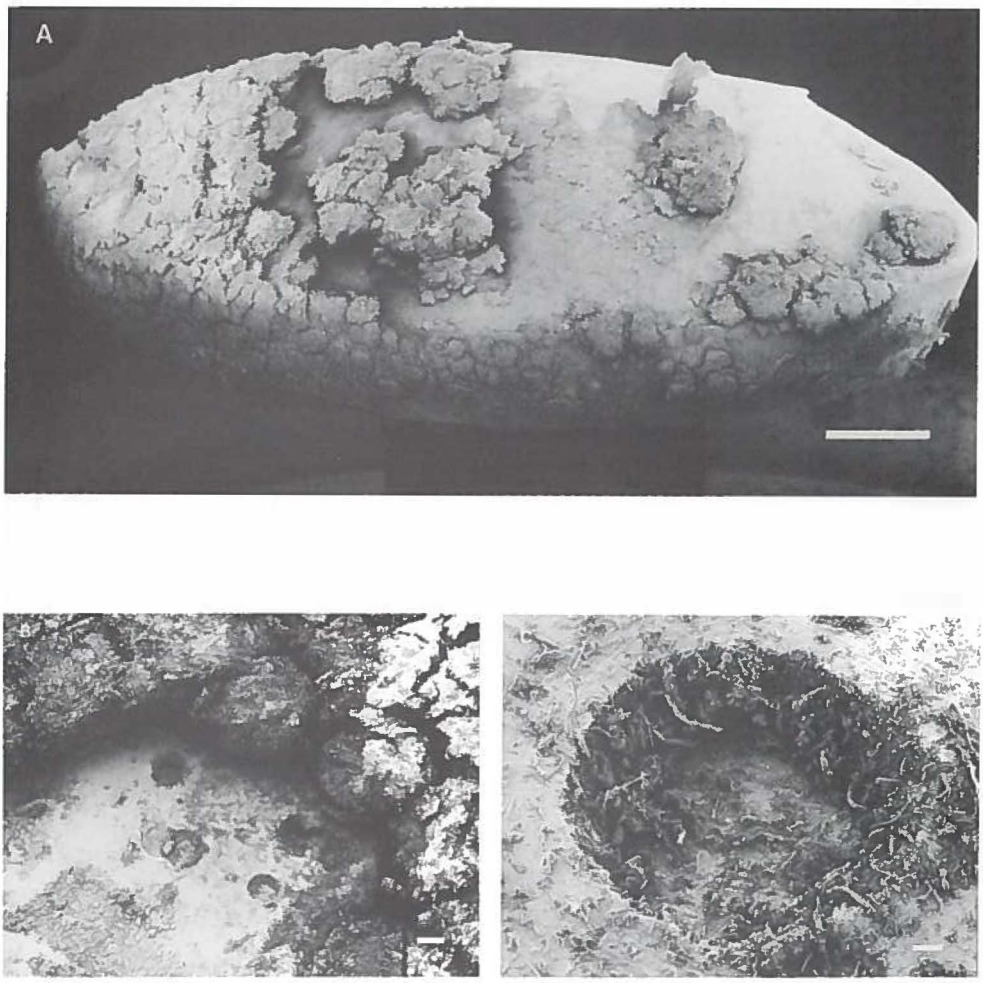


Figure 6. Scanning electron micrographs of partly hydrophilized "Groningen Button" voice prostheses removed from laryngectomized patients after a planned evaluation period of four weeks. A, low-magnification electron micrograph (bar equals 1 mm) showing differential biofilm formation on the hydrophilized (left) and original, hydrophobic side of (right) of the esophageal flange of a prosthesis. B, detail of a heavily colonized prosthesis (hydrophilized side) with ingrowing microorganisms, yielding deterioration of the silicone rubber surface. The bar denotes 100 μm. C, crater-like defect in the hydrophilized silicone rubber due to microbial ingrowth. The bar denotes 10 μm.

Table II. Microbial strains and species isolated from the original, hydrophobic (SR) and argon plasma treated (Ar), hydrophilized side of "Groningen Button" silicone rubber voice prostheses removed from laryngectomized patients, after a planned evaluation period of 4 weeks. "X" indicates detection of a strain or species.

	Patient A		Patient C		Patient D		Patient F		Patient G	
	SR	Ar	SR	Ar	SR	Ar	SR	Ar	SR	Ar
Yeast strains										
Candida albicans	X	X		X	X	X	X	X	X	X
Candida glabrata			X	X						
Candida inconspicua			X	X						
Candida krusei			X	X			X	X		
Candida lusitania			X	X						
Bacterial strains										
CDC group E (act. spp)			X		X		X			
Lactobacillus species					X	X	X	X		
Micrococcus luteus		X								
Salmonella subspecies				X						
Serratia marcescens	X	X								
Staphylococcal strains	X				X	X	X	X	X	X
Streptococcal strains	X	X					X	X		

hydrophobic valves are more or less identical. However, during high shear periods, the cohesivity of the conditioning film, which is strongly dependent upon the biomaterials hydrophobicity, may not be sufficient yielding detachment of the entire biofilm on top of it, as evidently occurs more readily on the hydrophobic side of a valve than on the hydrophilized side.

Nevertheless, this study convincingly demonstrates that biofilm formation on surfaces *in vivo* is governed by substratum hydrophobicity. For an improved anti-fouling performance of voice prostheses, increasing the hydrophobicity of the silicone rubber like e.g. by adsorption of fluorocarbons could be

a possibility. Fluorocarbon surfaces (i.e. Teflon) are slightly more hydrophobic than silicone rubber and hardly attracted any dental plaque during nine days exposure to dynamic conditions of the human oral cavity Quirynen *et al.*, 1989).

CONCLUSIONS

There are two main conclusions to be drawn from the results of this study:

1. Biofilm formation on voice prostheses in the oro-pharyngeal region is governed by the hydrophobicity of the surfaces exposed and occurs more readily on hydrophilic than on hydrophobic biomaterials surfaces, in contrast with expectations based on *in vitro* work.
2. *In vitro* studies on the fouling properties of biomaterials should be modified to include the dynamic conditions occurring *in vivo* in order to increase their predictive value with respect to the clinical performance of a biomaterial.

REFERENCES

Algaba J (1987), Récupération de la voix pour les patients laryngectomisés. *Revue de laryngologie* **108**:139-142.

Busscher HJ, de Boer CE, Verkerke GJ, Kalicharan R, Schutte HK and van der Mei HC (1994), *In vitro* ingrowth of yeasts into medical grade silicone rubber. *Int. Biodeterioration & Biodegradation.* **33**:383-390.

Busscher HJ, Bos R and van der Mei HC (1995), Initial microbial adhesion is a determinant for the strength of biofilm adhesion. *FEMS Microbiology Letters* **128**:229-234.

Busscher HJ and van der Mei HC (1995), Use of flow chamber devices and image analysis methods to study microbial adhesion. In: Methods in Enzymology, R.J. Doyle and I. Ofek (Eds), pp. 455-477. Academic Press, Inc., Florida.

Cooper JD, Pearson FG, Patterson GA, Todd TRJ, Ginsberg RJ Goldberg M and Waters P (1989), Use of silicone stents in the management of airway problems. *Ann. Thorac. Surg.* **47**:371-378.

Costerton JF, Nickel JC and Ladd TI (1986), Suitable methods for the comparative study of free-living and surface-associated bacterial populations. In: Bacteria in Nature, Vol. 2, Poindexter, J.S. Poindexter, E.R. Leadbetter (Eds), pp. 49-84. Plenum Press, New York.

Everaert EP, van der Mei HC, de Vries J and Busscher HJ (1995), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 1. Storage in air. *J. Adhesion Sci. Technol.* **9**:1263-1278.

Everaert EP, van der Mei HC and Busscher HJ (1996), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 2. A comparison of the hydrophobic recovery in air, water or liquid nitrogen. *J. Adhesion Sci. Technol.* **10**:351-359.

Everaert EPJM, Mahieu HF, Won Chung RP, Verkerke GJ, van der Mei HC and Busscher HJ (1997), A new method for *in vivo* evaluation of biofilms on surface modified silicone rubber voice prostheses. *Eur. Arch. Otorhinolaryngol.* **254**:261-263.

Gristina AG (1987), Biomaterial-centered infection. Microbial adhesion versus tissue integration. *Science* **237**:1588-1595.

Herrmann IF and Kley W (1981), Die glottoplastik mit einer ventil-prothese. *Arch Otorhinolaryngol.* **231**:647.

Hilgers FJM and Schouwenburg PF (1990), A new low-resistance, self-retaining prosthesis (Provox®) for voice rehabilitation after total laryngectomy. *Laryngoscope* **100**:1202-1207.

Hilgers FJM and Balm AJM (1993), Long term results of vocal rehabilitation after total laryngectomy with the low-resistance, indwelling Provox® voice prosthesis system. *Clin. Otolaryngol.* **18**:517-523.

Holmes AR, Cannon RD and Jenkinson HF (1995), Interactions of candida albicans with bacteria and salivary molecules in oral biofilms. *J. Ind. Microbiol.* **15**:208-213.

Izdebski K, Ross JC and Lee S (1987), Fungal colonization of tracheoesophageal voice prostheses. *Laryngoscope* 97:594-597.

Jenkinson HF, Lala HC and Shepherd MG (1990), Coaggregation of Streptococcus sanguis and other streptococci with candida albicans. *Infect. Immun.* **58**:1429-1436.

Kolenbrander PE (1988), Intergeneric coaggregation among human oral bacteria and ecology of dental plaque. *Ann. Rev. Microbiol.* **42**:627-656.

Mahieu HF, van Saene JJM, den Besten J (1986), Oropharynx decontamination preventing candida vegetation on voice prostheses. *Arch Otolaryngol Head Neck Surg.* **112**:1090-1092.

Mahieu HF, van Saene HKF, Rosingh, Schutte HK (1986), Candida vegetations on silicone voice prostheses. *Arch Otolaryngol Head Neck Surg.* **112**:321-325.

Mahieu HF, Annyas AA, Schutte HK and van der Jagt EJ (1987), Pharyngoesophageal myotomy for vocal rehabilitation of laryngectomies. *Laryngoscope* 97:451-457.

Mozes N, Léonard AJ and Rouxhet PG (1988), On the relation between the elemental surface composition of yeasts and bacteria and their charge and hydrophobicity. *Biochim. et Biophys. Acta* **945**:324-334.

Neu TR, van der Mei HC, Busscher HJ, Dijk F and Verkerke GJ (1993), Biodeterioration of medical-grade silicone rubber used for voice prostheses: a SEM study. *Biomaterials* **14**:459-464.

Neu TR, de Boer CE, Verkerke GJ, Schutte HK, Rakhorst G, van der Mei HC and Busscher HJ (1994), Biofilm development in time on a silicone voice prosthesis, a case study. *Microb. Ecol. in Health and Disease* 7:27-33.

Neu TR, Verkerke GJ, Herrmann IF, Schutte HK, van der Mei HC, Busscher HJ (1994), Microflora on explanted silicone rubber voice prostheses: taxonomy, hydrophobicity and electrophoretic mobility. *J. Appl. Bacteriology* 76:521-528.

Nijdam HF, Annyas AA, Schutte HK and Leever H (1982), A new prosthesis for voice rehabilitation after laryngectomy. *Arch. Otorhinolaryngol.* **237**:27-33.

Pitt WG, McBride MO, Barton AJ and Sagers RD (1993), Air-water interface displaces adsorbed bacteria. *Biomaterials* **14**:605-608.

Pratt-Terpstra IH, Weerkamp AH and Busscher HJ (1989), Microbial factors in a thermodynamic approach of oral streptococcal adhesion to human enamel and artificial substrata with various surface free energies. *J. Dent Res.* **68**:463-467.

Quirynen M, Marechal M, van Steenberghe D, Busscher HJ and van der Mei HC (1991), The bacterial colonization of intra-oral hard surfaces in vivo: influence of surface free energy and surface roughness. *Biofouling* **4**:187-198.

Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Arends J, Darius PL and van Steenberghe D (1989), The influence of surface free energy on planimetric plaque growth in man. *J. Dent. Res.* **68**:796-799.

Singer MI and Blom ED (1981), Selective myotomy for voice restoration after total laryngectomy. *Arch. Otolaryngol.* **107**:670-673.

Staffieri M and Staffieri A (1988), A new voice button for post-total laryngectomy speech rehabilitation. *Laryngoscope* **98**:1027-1029.

Traissac L, Devars F, Gioux M, Petit J, Benjebria A, Henry CH and Ucelay I (1987), La réhabilitation vocale du laryngectomisé par implant phonatoire. *Revue de laryngologie* **108**:157-159.

Van den Hoogen FJA, Oudes MJ, Janssen P, Hombergen G, Nijdam HF and Manni JJ (1996), The Groningen, Nijdam and Provox voice prostheses: a prospective clinical comparison based on 845 replacement. *Acta. Otolaryngol.* **116**:119-124.

Zijlstra RJ, Mahieu HF, Lith-Bijl JT and Schutte HK (1991) Aerodynamic properties of the low-resistance Groningen button. Arch. Otolaryngol. *Head Neck Surg.* **117**:657-661.

A NEW METHOD FOR IN VIVO EVALUATION OF BIOFILMS ON SURFACE-MODIFIED SILICONE RUBBER VOICE PROSTHESES

E.P.J.M. Everaert, H.F. Mahieu, R.P. Wong Chung, G.J. Verkerke,
H.C. van der Mei and H.J. Busscher*

ABSTRACT A new method is presented that permits a rapid and accurate in vivo evaluation of biofilm formation on surface modified silicone rubber voice prostheses. The method is based on partial modification of a Groningen button voice prosthesis by exposing half of the prosthesis to an argon plasma. This results in one side of the prosthesis becoming hydrophilic while leaving the unmodified side hydrophobic as a control. Modified prostheses were placed in patients for an evaluation period of approximately 4 weeks. Despite making the silicone rubber surface hydrophilic, biofilm formation was stimulated when compared to unmodified, hydrophobic silicone rubber. Findings show that biofilm formation on voice prostheses is influenced by hydrophobicity of a silicone rubber surface. The method of partial surface modification used was seen to be suitable for demonstrating such influences regardless of nutrition and other variations in patient's lifestyle. Microbiological analysis of the biofilms on both sides of the prosthesis valve did not show any changes in microbial composition, with *Candida albicans*, streptococci and staphylococci being the most commonly isolated strains.

INTRODUCTION

Voice rehabilitation after laryngectomy using voice prostheses is generally considered to be superior to esophageal speech for most patients (Lith-Bijl *et al.*, 1992). A major drawback, however, involves colonization of the prostheses within several weeks by a thick biofilm that consists of a variety of adhering yeast and bacterial strains that eventually cause leakage or blocking of the valve (Mahieu *et al.*, 1986; Neu *et al.*, 1993). As a consequence, indwelling silicone rubber voice prostheses, such as the Groningen button, Provox or Nijdam prostheses have to be replaced on average every 4 months (Van den Hoogen *et al.*, 1996).

Various attempts have been made to retard biofilm formation on indwelling voice prostheses, but with varying degrees of success. These have included having patients ingest large daily amounts of Turkish yoghurt or Kephir containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, use of a buccal bioadhesive slow-release tablet containing miconazole nitrate (Van Weissenbruch *et al.*, 1997) as well as selective decontamination of oropharyngeal yeast (Mahieu *et al.*, 1986). Since a major

*With permission of Springer Verlag, Germany. Eur Arch Otorhinolaryngol 254: 261-263 (1997).

part of microbial adhesion to voice prostheses is determined by its surface properties, an alternative method for preventing contamination could possibly involve a modification of its surface properties. Radiofrequency plasma treatment is now being used to alter the hydrophobicity of biomedical device surfaces, especially since it only affects the biomaterial's surface and not its mechanical properties (Hoffman, 1984). Recently, we have shown that repeated argon plasma treatment of silicone rubber yields a permanently hydrophilic surface, with a reduction in water contact angle from 115 to 15° (Everaert *et al.*, 1995). *In vitro* evaluation of argon plasma surface modified silicone rubber demonstrated reduced adhesion of *Staphylococcus epidermidis*, *Streptococcus salivarius* and *Candida tropicalis* when compared to unmodified silicone rubber. However, adhesion of *C. albicans* was not influenced. *In vitro* results are never a warranty for the clinical performance of a biomaterial and should be confirmed by results from *in vivo* evaluation. Unfortunately, *in vivo* comparisons of the fouling properties of different materials or surface modifications of a voice prosthesis are difficult, if not impractical, to conduct. The duration of the experiments necessary is too long and variations in patient's lifestyle and food consumptions over a time period exceeding 3-4 months cannot be adequately controlled.

The aim of this study was to present a new method allowing a rapid and accurate *in vivo* evaluation of biofilm formation on surface-modified silicone rubber voice prostheses as compared to biofilm formation on unmodified prostheses. Additionally, the clinical performance of argon plasma-treated, hydrophilized silicone rubber for use as a voice prosthesis was evaluated.

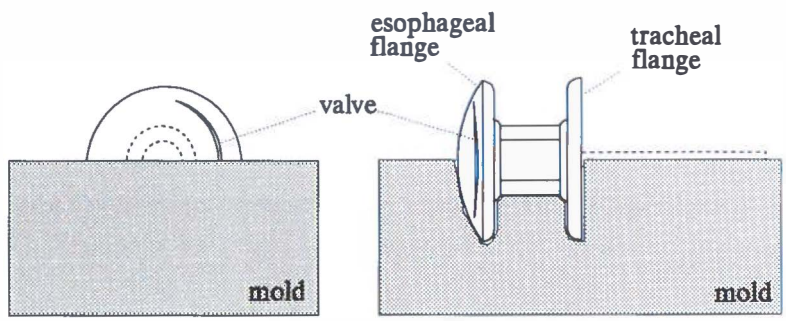


Figure 1 *Schematic drawing of a Groningen button voice prosthesis placed in a plaster cast to shield half of the prosthesis from argon plasma while making the other half hydrophilic.*

MATERIALS AND METHODS

"Low Resistance" and "Ultra-Low Resistance" Groningen button voice prostheses were provided by Medin Instruments and Supplies (Groningen, The Netherlands) and were exposed in part to an argon plasma by placing the silicone rubber prostheses in a plaster cast (Dental superhart gypsum, New Fujirock, GC Corporation, Tokyo, Japan) prior to insertion in a plasma chamber (Fig. 1). The plasma treatment was repeated six times with a 1-day interval in between. This produced prostheses with a permanently hydrophilic side and with an unmodified, hydrophobic side (Everaert *et al.*, 1995; 1996), as can be seen in Fig. 2.

Seven laryngectomized patients with at least 6 months experience with a voice prosthesis were given a partly treated Groningen button voice prosthesis for a planned evaluation period of approximately 4 weeks. After removal of the voice prosthesis from the tracheo-esophageal shunt, biofilm formation on the modified and unmodified sides of the prosthesis were compared by light and scanning electron microscopy. Microbial compositions of the biofilms on both sides of the valves were compared by plating on brain-heart infusion and blood agar plates at 37°C under aerobic conditions (Neu *et al.*, 1994) to determine possible shifts in microbial composition due to the surface modifications.

RESULTS

Partial and full argon plasma treatment of the Groningen button voice prostheses did not change air-flow resistances (Rhinomanometer, Mercury NR3), nor was the biocompatibility of the silicone rubber according to an approved Agar diffusion test (BSC105/2, Bioscan BV, Bilthoven, The Netherlands) adversely affected.

All prostheses removed from the tracheo-esophageal shunts showed differences in biofilm formation on their hydrophilic and hydrophobic sides, with the hydrophilic side being more apt to biofilm formation. Examples of light microscopic findings of the esophageal sides of two prostheses are shown in Fig. 3, with the borderline between the modified and unmodified sides clearly visible from the differential fouling. A scanning electron microscopic study of a partly modified prosthesis (Fig. 4) clearly shows enhanced biofilm formation on the hydrophilic side as compared to the hydrophobic side.

Microbial analysis of the biofilms on both sides revealed no shift in microbial composition of the biofilms as a result of the surface modification: i.e., prostheses from the 7 laryngectomized patients studied contained similar microbial strains and species from both sides of the prostheses. Commonly isolated strains were *C. albicans*, streptococci and staphylococci.

Figure 2. *Photograph of a silicone rubber Groningen button, partly modified in argon plasma to produce a hydrophilic side on which water droplets spread well (right side, with a low water contact angle). The unexposed silicone rubber side is left hydrophobic (left side, high water contact angle). The bar represents 2.0 mm.*

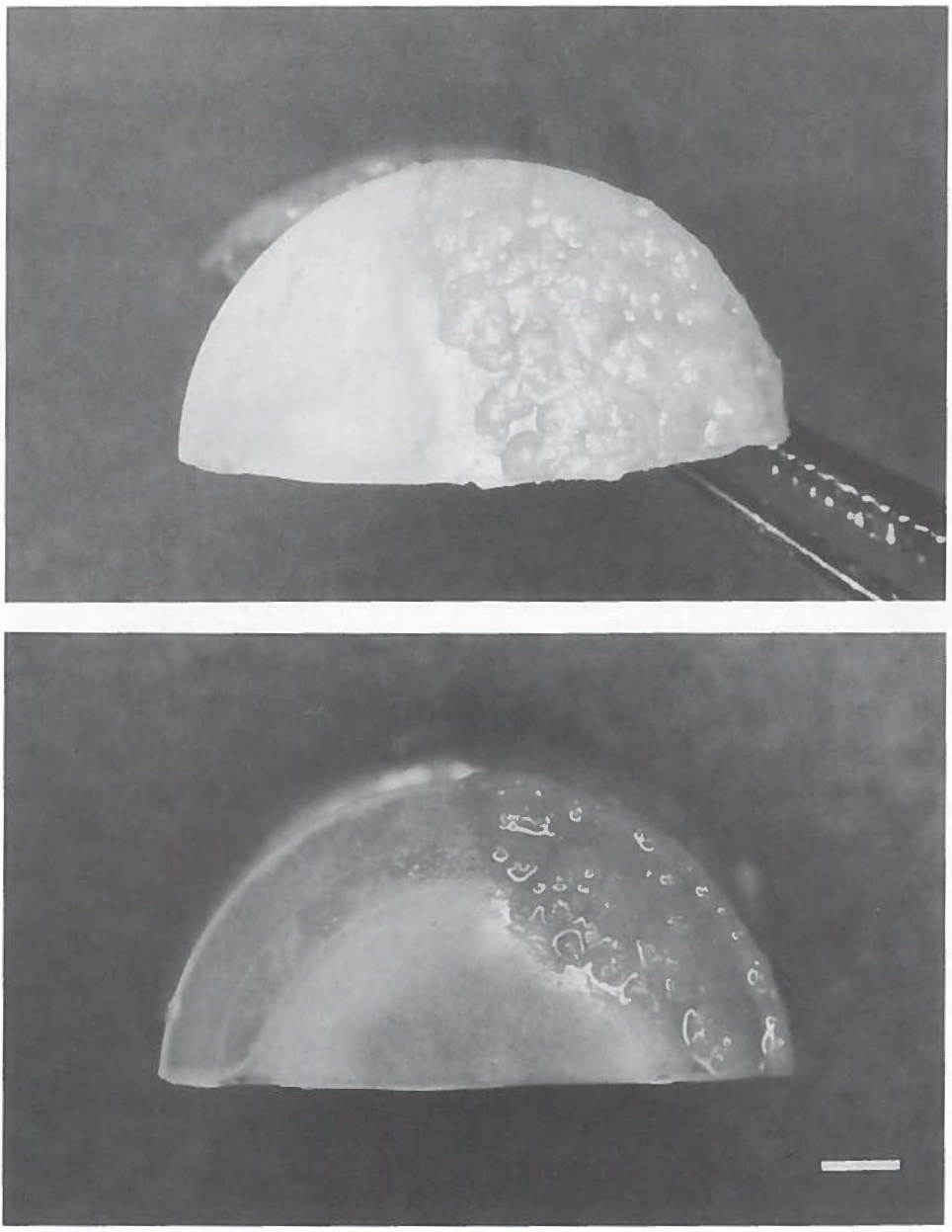


Figure 3. *Light micrographs of two partly surface-modified Groningen button voice prostheses after 4 weeks in situ demonstrating influence of the surface modification on biofilm formation. The right side of each voice prosthesis is the surface modified side, while the left side is the unmodified side. The bar represents 2.0 mm.*

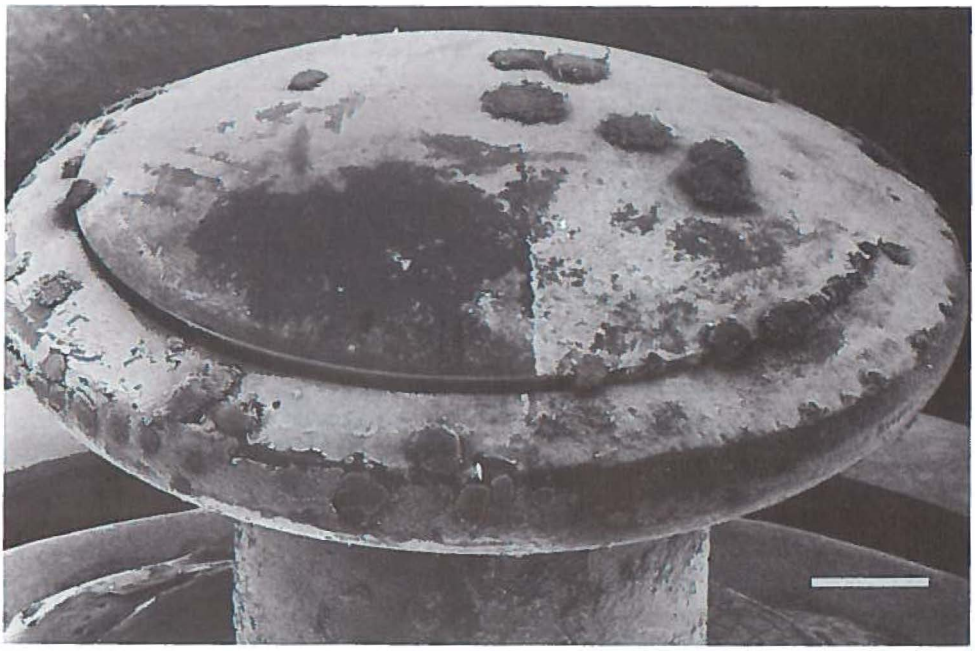


Figure 4. *Scanning electron micrographs of a Groningen button voice prosthesis removed from a tracheo-esophageal shunt, of which the right side was made hydrophilic prior to placing the prosthesis in a patient for a 4-week evaluation period. The bar represents 2.0 mm.*

COMMENT

Despite finding that the argon plasma modification did not reduce biofilm formation on the Groningen button voice prostheses tested, our study clearly shows that the hydrophobicity of the silicone rubber surface influenced fouling of the prostheses *in vivo*. Furthermore, the use of partly surface modified prostheses, whether by plasma treatments or other wet chemical modifications, appeared very suitable to demonstrate such influences over a reasonably short evaluation period. We believe that this was due to the exclusion of nutritional and other patient lifestyle-related factors.

REFERENCES

Everaert EPJM, van der Mei HC, de Vries J, Busscher HJ (1995), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 1. Storage in air. *J. Adhesion Sci. Technol.* 9:1263-1278.

Everaert EPJM, van der Mei HC, Busscher HJ (1996), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 2. A comparison of the hydrophobic recovery in air, water or liquid nitrogen. *J. Adhesion Sci. Technol.* **10**:351-359.

Hoffman AS (1984), Ionizing radiation and gas plasma (or glow) discharge treatments for preparation of novel polymeric biomaterials. In: Dusek K (ed) Polymers in medicine, 57. Advances in polymer science. Springer, Berlin Heidelberg New York, pp 141-157.

Lith-Bijl JT, Mahieu HF, Patel P, Zijlstra RJ (1992), Clinical experience with the low-resistance Groningen button. *Eur. Arch. Otorhinolaryngol.* **249**:354-357.

Mahieu HF, van Saene HKF, Rosingh HJ, Schutte HK (1986), *Candida* vegetations on silicone voice prostheses. *Arch. Otolaryngol. Head Surg.* **112**:321-325.

Mahieu HF, van Saene JJM, den Besten J, van Saene HKF (1986), Oropharynx decontamination preventing *Candida* vegetation on voice prostheses. *Arch. Otolaryngol Head Neck Surg.* **112**:1090-1092.

Neu TR, van der Mei HC, Busscher HJ, Dijk F, Verkerke GJ (1993), Biodeterioration of medical-grade silicone rubber used for voice prostheses: a SEM study. *Biomaterials* **14**:459-464.

Neu TR, Verkerke GJ, Herrmann IF, Schutte HK, van der Mei HC, Busscher HJ (1994), Microflora on explanted silicone rubber voice prostheses: taxonomy, hydrophobicity and electrophoretic mobility. *J Appl Bacteriol.* **76**:521-528.

Van den Hoogen FJA, Oudes MJ, Janssen P, Hombergen G, Nijdam HF, Manni JJ (1996), The Groningen, Nijdam and Provox Voice Prostheses: a prospective clinical comparison based on 845 replacement. *Acta Otolaryngol.* (Stockh) **116**:119-124.

Van Weissenbruch R, Bouckaert S, Remon J-P, Nelis HJ, Aerts R, Albers FWJ (1997), Chemoprophylaxis of fungal deterioration of the Provox silicone tracheoesophageal prosthesis in postlaryngectomy patients. *Ann. Otol. Rhinol. Laryngol.* **106**:329-337.

ADHESION OF YEASTS AND BACTERIA TO FLUORO-ALKYLSILOXANE LAYERS CHEMISORBED ON SILICONE RUBBER

E.P.J.M. Everaert, H.C. van der Mei and H.J. Busscher*

ABSTRACT Indwelling voice prostheses are most often made of silicone rubber. However, the silicone rubber surface attracts huge quantities of adhering yeasts and bacteria and their colonization on the valve side of voice prostheses leads to frequent malfunctioning. On an average, indwelling voice prostheses have to be replaced every 3-4 months. In this paper, we report on the *in vitro* adhesion of yeasts and bacteria to fluoro-alkylsiloxane layers chemisorbed on silicone rubber surfaces, as measured in a parallel plate flow chamber. Silicone rubber surfaces were first oxidized with an argon plasma treatment (Ar-SR). In a second step, organic layers were created by chemisorption of fluoro-alkyltrichlorosilanes onto the Ar-SR surfaces, denoted as Ar-SR-CF_3 and Ar-SR-C_8F_{17}, respectively. Physico-chemical properties of the chemisorbed layers were studied by water contact angle measurements, X-ray photoelectron spectroscopy (XPS) and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Using a parallel plate flow chamber, adhesion of *Streptococcus salivarius*, *Staphylococcus epidermidis*, *Candida albicans* and *Candida tropicalis* strains, isolated from explanted voice prostheses, was investigated to the chemisorbed fluoro-alkylsiloxane layers with and without a salivary conditioning film. Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces showed significantly reduced microbial adhesion as compared to original silicone rubber, both with respect to initial deposition rates as well as with respect to adhesion in a stationary end-point. Furthermore, adhering microorganisms were more easily detached when applying an air-liquid interface. Silicone rubber surfaces with chemisorbed, long fluorocarbon chains (Ar-SR-C_8F_{17}) showed the greatest reduction in microbial adhesion, probably because of their low surface free energy combined with a higher surface entropy.

INTRODUCTION

The prolonged use of indwelling silicone rubber voice prostheses by laryngectomized patients is limited to 3-4 months on an average (van den Hoogen *et al.*, 1996). Thick biofilms, consisting of a variety of oral and skin microorganisms amongst which streptococci, staphylococci and yeasts, on the valve side of the prostheses either cause leakage or increased air-flow resistance (Mahieu *et al.*, 1986; Neu *et al.*, 1993). Especially adhesion of yeasts is troublesome, as they have the tendency to grow into

*Submitted to Colloids and Surfaces B:Biointerfaces

the silicone rubber (Neu *et al.*, 1993; Busscher *et al.*, 1994). Therewith their detachment by naturally occurring shear forces in the oropharyngeal cavity is impeded.

From a physico-chemical point of view, it has been documented that microbial adhesion to substratum surfaces is an interplay of the hydrophobic and electrostatic properties of the interacting surfaces (Absolom, 1988; Busscher and Weerkamp, 1987; Mozes *et al.*, 1988). Under *in vivo* conditions, the adhesion process is complicated by the formation of a conditioning film prior to the adhesion of the first arriving microorganisms (Gristina, 1987), that masks the surface properties of the substratum surface (Schneider and Marshall, 1994; Van Dijk *et al.*, 1988). In the oropharyngeal cavity, the conditioning film is predominantly of salivary origin and known to consist for the major part of adsorbed salivary proteins. The composition (van der Mei *et al.*, 1993), structure and cohesive strength of salivary conditioning films in the oropharyngeal cavity depends on the hydrophobicity of the underlying substratum (Busscher *et al.*, 1995). Conditioning films on hydrophobic surfaces have a low cohesive strength, as biofilms on such conditioning film easily detach, presumably through cohesive failure in the conditioning film under the fluctuating shear forces operative. Oppositely, conditioning films on hydrophilic surfaces are suggested to have much higher cohesive strength. Oral streptococci, for instance, adhere in roughly equal numbers to salivary conditioning films on hydrophobic and hydrophilic substrata *in vitro* (Pratt-Terpstra *et al.*, 1989), but when glued on the front incisors of human volunteers hydrophobic substrata collect far less dental biofilm than hydrophilic substrata, as evaluated after nine days *in vivo* (Quirynen *et al.*, 1991). A similar observation with important implications for the development of an anti-fouling coating on silicone rubber voice prostheses has recently been done evaluating biofilm formation, *in vivo*, on partly hydrophilized silicone rubber voice prostheses (Everaert *et al.*, in press 1997). Even after four weeks in the oropharyngeal cavity of laryngectomized patients, the hydrophilized side of silicone rubber valves had collected a much more extensive biofilm than the authentic, hydrophobic silicone rubber side (Everaert *et al.*, in press 1997). As *in vitro*, microorganisms adhered both to hydrophobic as well as to hydrophilized silicone rubber surfaces (Everaert *et al.*, submitted 1996), it is concluded that detachment of adhering microorganisms may be more relevant to study under *in vitro* conditions to mimic the *in vivo* situations than adhesion. Interestingly, also hydrophilizing surfaces in the oropharyngeal cavity shows promising for the control of biofilm formation, provided the hydrophilicity is created by long, high entropy polymer chains (Olsson *et al.*, 1991).

Considering that even, or better "especially", under clinical conditions in the oropharynx, biofilm formation is governed by substratum hydrophobicity, the aim of this work is to increase the hydrophobicity of silicone rubber surfaces in order to reduce microbial adhesion. To this end, reactive silicone rubber surfaces were prepared by argon plasma glow discharge prior to anchoring fluoro-alkyltrichlorosilanes. Subsequently, a detailed physico-chemical analysis of the surfaces was made by

contact angle measurements, X-ray photoelectron spectroscopy and attenuated total reflection Fourier transform infrared spectroscopy after which adhesion and detachment of two bacterial and two yeast strains to modified silicone rubber substrata was studied in a parallel plate flow chamber.

EXPERIMENTAL

Silicone rubber and surface modification

Silastic Implant Silicone Rubber, a dimethyl and methylvinyl siloxane copolymer, (MED-4750, NuSil Silicone Technology, Antwerpen, Belgium) kit was purchased. Plates 0.5-mm-thick 50 x 76 mm were produced following the procedures suggested by the manufacturer. Briefly, equal proportions of part A and B were thoroughly blended together and injected into a mold at room temperature. Subsequently, the silicone rubber was immediately cured at 200°C for 50 min. Finally, samples were cleaned in a 2% RBS 35 (Omnilabo International B.V., Breda, The Netherlands) detergent solution under simultaneous sonication (5 min, 150 W) and thoroughly rinsed in Millipore® grade water and absolute ethanol (>96%).

Silicone rubber surfaces have been modified in a two-step process. In the first step, the silicone rubber surfaces were oxidized once in an argon plasma (Ar-SR) as described before (Everaert *et al.*, 1995). In the second step, one of the two fluoro-alkyltrichlorosilanes (Fluka Chemie AG, Buchs, Switzerland) CF_3-$(CH_2)_2$-$SiCl_3$ and CF_3-$(CF_2)_7$-$(CH_2)_2$-$SiCl_3$ were chemisorbed onto Ar-SR surfaces, denoted as Ar-SR-CF_3 and Ar-SR-C_8F_{17}, respectively. To this end, silane compounds were diluted in perfluoroheptane (Fluka Chemie AG, Buchs, Switzerland) to a final concentration of 0.5 %. Subsequently, Ar-SR surfaces were put into these solutions for 10 min. Perfluoroheptane was chosen because this solvent does not swell the silicone rubber. Finally, silane-treated surfaces were washed with fresh perfluoroheptane, and absolute ethanol.

Contact angle measurements, X-ray photoelectron and Fourier transform infrared spectroscopy

Water contact angles were measured on the silicone rubber samples by the sessile drop technique. The advancing and receding water contact angles were obtained by placing the needle in the water droplet (1 - 1.5 µl) and carefully moving the sample until the advancing angle appeared to be maximal. Contact angles with the liquid droplet at rest will be referred to as equilibrium contact angles. All contact angles were calculated from droplet profiles determined with a home-made contour monitor. On each separately prepared sample surface, at least ten droplets were placed over different parts of the sample surface, yielding on average standard deviation of 2 and 3 degrees in advancing and receding contact angles, respectively.

XPS was performed using a S-Probe spectrometer (Surface Science Instruments, Mountain View,

CA, USA), as previously described (Everaert *et al.*, 1995). Binding energies were determined by setting the binding energy of the C_{1s} at 284.8 eV (Beamson and Briggs, 1992). Elemental surface compositions were expressed in atomic %, setting % C + % O + % Si + % F to 100%.

ATR-FTIR absorption spectra were recorded on a FTS-175 spectrometer from Bio-Rad Laboratories (USA) equipped with a Golden Gate™ Single Reflection Diamond ATR (10500 series, Graseby Specac, Fairfield, CT, USA). The spectral resolution and wave number accuracy were 2 cm^{-1} and 0.01 cm^{-1}, respectively. All measurements consisted of 100 scans, using air as background reference.

Microbial strains and growth conditions

The bacterial and yeast strains used in this study were isolated from "Groningen Button" voice prostheses (Neu *et al.*, 1994), removed from patients complaining either about leakage or blocking of the valve and included two bacterial strains *Streptococcus salivarius* GB 24/9, cultured in Todd Hewitt broth, *Staphylococcus epidermidis* GB 9/6, cultured in Tryptone Soya broth and the two yeasts strains (*Candida albicans* GB 1/2 and *Candida tropicalis* GB 9/9) cultured in Brain Heart Infusion broth. Culture media were purchased from Oxoid, Unipath LTD, Basingstoke, Hampshire, England. All microorganisms were inoculated from agar plates into a batch culture for 24 h at 37°C in ambient air, which was used to inoculate a second culture which was grown for 16 h under similar conditions.

The microorganisms were harvested by centrifugation (5 min at 4,000 *g* for bacterial and 10,000 *g* for the yeast strains), washed twice with Millipore® water and resuspended in adhesion buffer (50 mM KCl, 2 mM potassium phosphate and 1 mM $CaCl_2$, pH 6.8), bacteria to a concentration of 3 x 10^8 per ml and yeasts to a concentration of 3 x 10^6 per ml, as determined in a Bürker-Türk counting chamber.

Saliva

From healthy volunteers of both sexes, human whole saliva was collected into ice-chilled cups. Saliva was stimulated by the volunteers chewing Parafilm®. After the saliva was pooled and centrifuged at 10,000 *g* for 10 min at 4°C, phenylmethylsulfonylfluoride (0.2 M) was added to a final concentration of 1 mM as a protease inhibitor. The solution was again centrifuged, dialysed for 48 h at 4°C against Millipore® water and freeze dried for storage. A solution of 1.5 $mg.ml^{-1}$ of freeze dried stock in adhesion buffer will be denoted as (reconstituted human whole) saliva.

The parallel plate flow chamber and image analysis

The flow chamber and image analysis system have been previously described (Busscher and van der Mei, 1995). Images were taken from the bottom plate of the parallel plate flow chamber which

consisted of a silicone rubber or treated silicone rubber sample affixed to a thicker (1.5 mm) perspex plate. The top plate of the chamber was made of glass.

Deposition was observed with a CCD-MXRi camera (High Technology, Eindhoven, The Netherlands) mounted on a phase contrast microscope (Olympus BH-2) equipped with a 40 x ultra long working distance objective (Olympus ULWD-CD Plan 40 PL) for experiments with bacteria and with a 10 x objective for experiments with yeasts. The camera was coupled to an image analyzer (TEA, Difa, Breda, The Netherlands). Each image (512 x 512 pixels with 8 bits resolution), obtained after summation of 8 consecutive images (time interval 1 sec) in order to enhance the signal-to-noise ratio and to eliminate moving microorganisms from the analysis. Subsequently, adhering microorganisms were discriminated from the background by a single grey-value threshold yielding a binary black and white image and the number of adhering microorganisms was counted. An image covers a surface area of 0.017 mm^2 at the magnification used for bacterial experiments and 0.3 mm^2 at the magnification employed in the experiments with yeasts.

Prior to each experiment, all tubes and the flow chamber were filled with adhesion buffer, while care was taken to remove air bubbles from the system. Flasks, containing microbial suspension, buffer and saliva when appropriate, were positioned at the same height with respect to the chamber to ensure that immediately after the flows were started, all fluids would circulate through the chamber at the desired shear rate of 10 s^{-1} (0.025 $ml.s^{-1}$), which yields a laminar flow (Reynolds number 0.6).

When a salivary conditioning film was required, flow was switched first to saliva for 1.5 h, followed by a flow of freshly made buffer during 1 h to remove all remnants of saliva from the tubes and chamber. The microbial suspension was circulated through the system for 4 h and images were obtained at the highest possible rate. The initial increase in the number of adhering microorganisms with time was expressed in a so-called initial deposition rate j_0 [$cm^{-2}.s^{-1}$], i.e. the number of microorganisms adhering per unit time and area. The number of microorganisms adhering after 4 h, n_{4h} was taken as an estimate of microbial adhesion in a more advanced state of the process. Finally, while focus was maintained on the same spot of the substratum, the number of adhering microorganisms in this field of view was compared with the number of microorganisms that remained adhering after passing an air-bubble through the chamber to obtain an indication of the adhesive forces (Leenaars and O'Brien, 1989; Pitt *et al.*, 1993).

All microbial adhesion data given in this paper are the means of experiments with two separately cultured microbial suspensions and individually prepared silicone rubber substrata.

RESULTS

Water contact angles

Table I summarizes the advancing, receding and equilibrium water contact angles on untreated, Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces. Silicone rubber surfaces are already relatively hydrophobic with an advancing water contact angle of 115° and a moderate contact angle hysteresis of 31°. Chemisorption of fluoro-alkyltrichlorosilanes increases the hydrophobicity of the silicone rubber surfaces to advancing water contact angles of 125° and 140° for Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces, respectively. The water contact angle hysteresis is largest (45°) for Ar-SR-C_8F_{17} surfaces as compared to Ar-SR-CF_3 surfaces (35°).

Table I. Advancing(θ_A), receding (θ_R) and equilibrium (θ_E) water contact angles of untreated silicone rubber (SR), Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces. All angles are given in degrees with ± indicating the SD over four separately prepared samples

Sample	θ_A	θ_R	θ_E
SR	115±3	84±5	108±2
Ar-SR-CF_3	125±5	90±6	114±5
Ar-SR-C_8F_{17}	140±5	95±7	125±5

Elemental surface composition

In Table II, the elemental composition of silicone rubber with and without chemisorbed fluoro-alkyltrichlorosilanes are summarized. Incidentally, it is noted that we were not able to chemisorb monochlorosilanes to bare silicone rubber, while also their chemisorption to argon plasma treated silicone rubber was relatively low. Trichlorosilanes were significantly more reactive than monochlorosilanes and could be more effectively coupled to the silicone rubber after argon plasma. Upon argon plasma treatment, the amount of surface fluor after CF_3-$(CH_2)_2$-$SiCl_3$ chemisorption increased from 11.3 to 33.5 %, while after CF_3-$(CF_2)_7$-$(CH_2)_2$-$SiCl_3$ chemisorption an increase from 50.5 to 62.4 % was observed. Chlorine was never detected by XPS.

More information on the structure of Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces can be obtained from the decomposition of the C_{1s} binding energies as shown in Fig. 1. The C_{1s} peak of untreated silicone rubber has two components at 284.5 and 285.6 eV for carbon involved in methyl groups of the siloxane bond and in crosslinking between the siloxane chains, respectively (Eveaert *et al.*, 1996).

Table II. XPS elemental composition of fluoro-alkyltrichlorosilanes[a] adsorbed onto silicone rubber (SR) and argon plasma-treated silicone rubber surfaces (Ar-SR).

Sample	Atomic percentage			
	C_{1s}	O_{1s}	Si_{2p}	F_{1s}
SR	49.7	25.9	24.8	-
SR + CF_3-$(CH_2)_2$-$SiCl_3$	41.9	25.2	21.1	11.3
Ar-SR + CF_3-$(CH_2)_2$-$SiCl_3$	35.4	18.6	12.5	33.5
theoretical[b]	35.3	17.6	11.7	35.3
SR + CF_3-$(CF_2)_7$-$(CH_2)_2$-$SiCl_3$	34.4	7.8	7.3	50.5
Ar-SR + CF_3-$(CF_2)_7$-$(CH_2)_2$-$SiCl_3$	29.1	5.2	3.3	62.4
theoretical[c]	33.8	5.1	3.4	57.7

[a] in a separate study (unpublished), it was established that monochlorosilanes did not chemisorb to silicone rubber, while their adsorption to argon plasma treated silicone rubber was 5-10 times less than of trichlorosilanes, as concluded from F_{1s} electron counts in XPS.

[b] theoretical XPS composition based on the corresponding atomic structure with C_3 F_3 Si $O_{1.5}$ and [c] C_{10} F_{17} Si $O_{1.5}$ = 100 %, respectively (see Fig. 1).

The C_{1s} peak of Ar-SR-CF_3 surfaces has 3 components at 284.8, 286.0 and 292.6 eV corresponding to the carbon involved in -*C*H_2-Si(O-)$_3$, in -*C*H_2-CF_3 and in -CH_2-*C*F_3 bonds, respectively. In addition, the relative percentages of these three C_{1s} components are almost equivalent as expected on the basis of the molecular structure. The C_{1s} binding energy of Ar-SR-C_8F_{17} surfaces contains 6 components, consistent with the molecular structure of C_8F_{17}-$(CH_2)_2$-Si(O-)$_3$ layers chemisorbed on Ar-SR (see Fig. 1).

ATR-FTIR band assignments

The ATR-FTIR spectra of pure fluoro-alkyltrichlorosilanes, of Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces, and of untreated silicone rubber surfaces are given in Fig. 2. Table III, lists the observed bands and their assignments. Interestingly, the ATR-FTIR spectra of Ar-SR-CF_3 and Ar-SR-C_8F_{17} are consistent

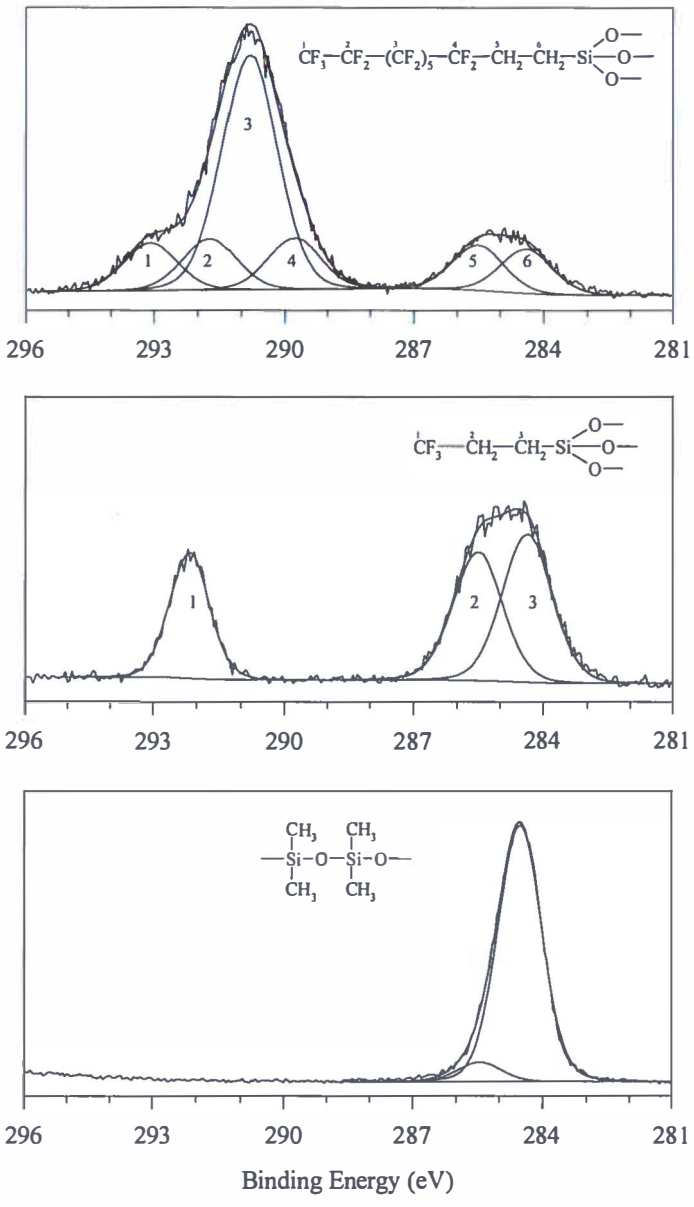


Figure 1. *C_{1s} binding energies of Ar-SR-C_8F_1-, Ar-SR-CF_3 surfaces and of untreated silicone rubber, with the carbon atoms involved in different bonds according to the decomposition indicated.*

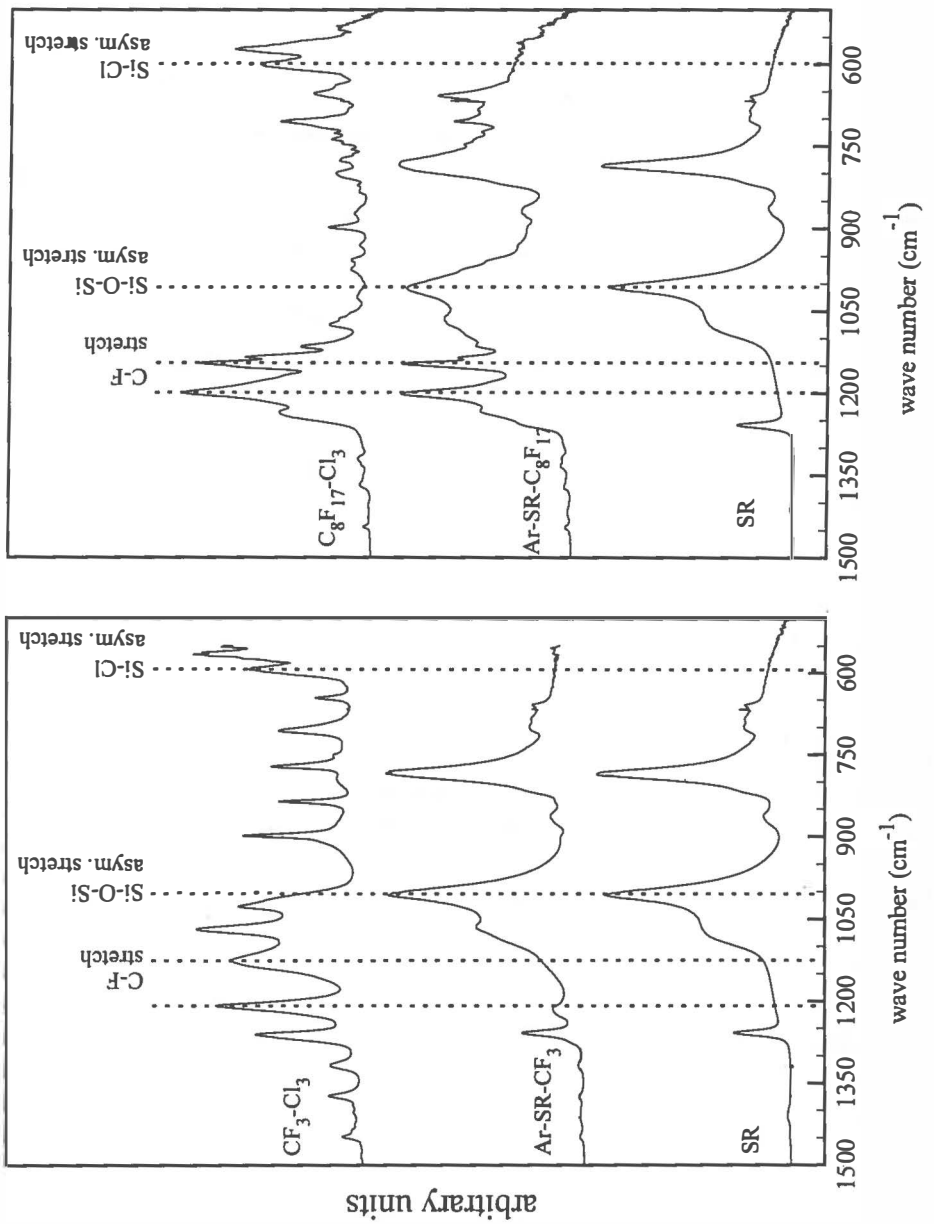


Figure 2. *ATR-FTIR spectra in the wave number region from 1500 to 500 cm^{-1} of untreated silicone rubber (SR), Ar-SR-CF_3 and the pure CF_3-$(CH_2)_2$-$SiCl_3$ molecule (CF_3-Cl_3), and of untreated silicone rubber (SR), Ar-SR-C_8F_{17} and the pure C_8F_{17}-$(CH_2)_2$-$SiCl_3$ molecule (C_8F_{17}-Cl_3). The most prominent bands are indicated, while a complete band assignment is given in Table III.*

with the chemical reaction occurring between the respective silane molecule and an Ar-SR surface. The Si-Cl asymmetric and symmetric stretch bands in the region 600-565 cm^{-1} of the silanes clearly disappear after chemisorption to the argon plasma treated silicone rubber (see Fig. 2).

Table III. Assignment of the ATR-FTIR bands in the wavenumber region between 1500 and 500 cm^{-1} of silicone rubber and of pure fluoro-alkyltrichlorosilanes (see ATR-FTIR spectra in Fig. 2)

Adsorption band position (cm^{-1})	Assignment
SR	
1258	CH_3 symmetric deformation in Si-CH_3
1060,1006	Si-O-Si asymmetric stretch
862	CH_3 rocking in Si-CH_3
786	Si-O-Si symmetric stretching
CF_3-$(CH_2)_2$-$SiCl_3$	
1449	-CH_2- scissoring
1373, 1318, 1208	CF_3- stretch
1125, 1069, 1027	CF_3- stretch
837, 772, 708	CF_3- symmetric deformation
648	CF_3- asymmetric deformation
591	Si-Cl asymmetric stretch
566	Si-Cl symmetric stretch
C_8F_{17}-$(CH_2)_2$-$SiCl_3$	
1428	-CH_2- scissoring
1370, 1320, 1237	C-F stretch
1200, 1145, 1134	C-F stretch
897, 705	C-F symmetric deformation
653	CF_3- asymmetric deformation
598	Si-Cl asymmetric stretch
572	Si-Cl symmetric stretch

Microbial adhesion

Microbial adhesion, including the initial deposition rate j_0, the adhesion in a stationary end point n_{4h} and the percentage of adhering microorganisms detached after the passage of an air-bubble through the flow chamber, are compiled in Figs. 3 and 4 for the bacterial and yeast strains, respectively. As can be seen from Figs. 3 and 4, initial deposition rates and the number of adhering microorganisms after 4 h to Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces were always lower than to untreated silicone rubber both in the absence and presence of a salivary conditioning film. Moreover, all strains were more readily detached by the passage of an air-liquid interface from the chemisorbed fluoro-alkylsiloxane layers than from untreated silicone rubber. By comparison, it can be seen that Ar-SR-C_8F_{17} layers are less adhesive for bacteria and yeasts than Ar-SR-CF_3 surfaces.

DISCUSSION

Chemisorption of fluoro-alkyltrichlorosilanes to silicone rubber could be best achieved by first treating the silicone rubber with an argon plasma, therewith introducing reactive polar groups, as schematically indicated in Fig. 5. Subsequently, chlorosilane molecules can react with these polar groups to form siloxane bonds. The effective reaction of the chlorosilane with argon plasma treated silicone rubber is evidenced by the lack of the Si-Cl stretching bands in the ATR-FTIR spectra of the Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces and the absence of chlorine as measured by XPS. Trichlorosilane molecules not only react with the argon plasma treated silicone rubber, but also have the ability to polymerize, yielding the formation of relatively thick siloxane layers. Presumably, the siloxane layers created are at least as thick as the information depth of XPS analysis, i.e. 5-10 nm, as the chemical composition data of Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces are fully concurrent with theoretically calculated data (see Table II), demonstrating that no electrons are detected from the underlying silicone rubber. The contact angle hysteresis on the Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces is appreciable and indicate that the molecules in the siloxane layers have a high degree of freedom to reorient themselves in an aqueous environment versus air. The surface mobility of the Ar-SR-C_8F_{17} surfaces is probably larger than of the Ar-SR-CF_3 surfaces, as concluded from their larger water contact angle hysteresis. Considering the above, we propose a hypothetical structure for the chemisorption of fluoro-trichlorosilanes to argon plasma treated silicone rubber, consisting of a relatively thick, dendritic wedge structure with, especially for the long chain fluoro-siloxanes, a reasonable degree of freedom of the polymer chains (see Fig. 5). Comparatively, Silver and co-workers (1995) chemisorbed C_8F_{17}-$(CH_2)_2$-$SiCl_3$ onto oxygen plasma treated silicone rubber and only found 11 % surface fluorine, indicating a much thinner fluoro-alkylsiloxane network than created after argon plasma treatment.

The hydrophobicity of silicone rubber is hard to increase and in a recent study on the basis of

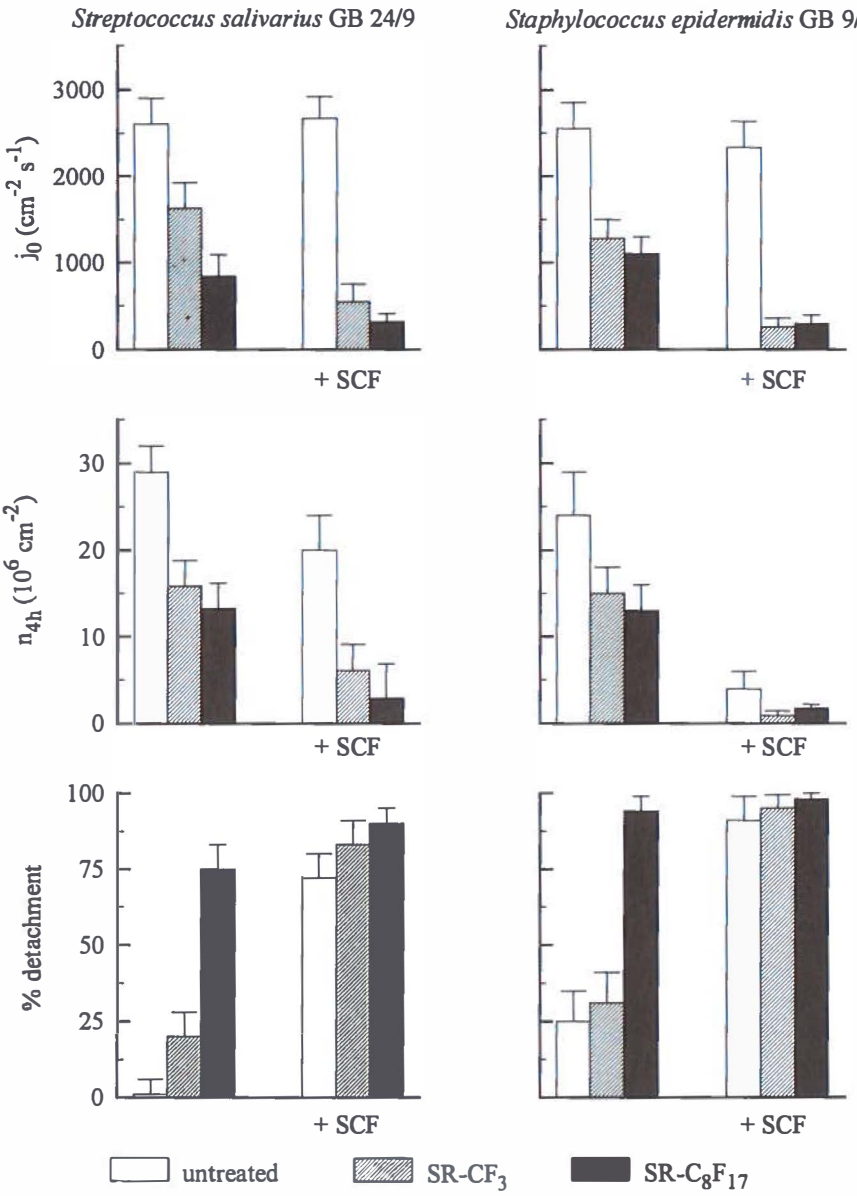


Figure 3. *Initial deposition rates j_0, numbers of adhering bacteria after 4 hours n_{4h}, and percentages of bacteria detached after passing an air-bubble through the flow chamber to Ar-SR-CF_3 and Ar-SR-C_8F_{17} surfaces and untreated silicone rubber, in the absence and presence of a salivary conditioning film (SCF) for two bacterial strains; S. salivarius GB 24/9 (left hand panel) and S. epidermidis GB 9/6 (right hand panel).*

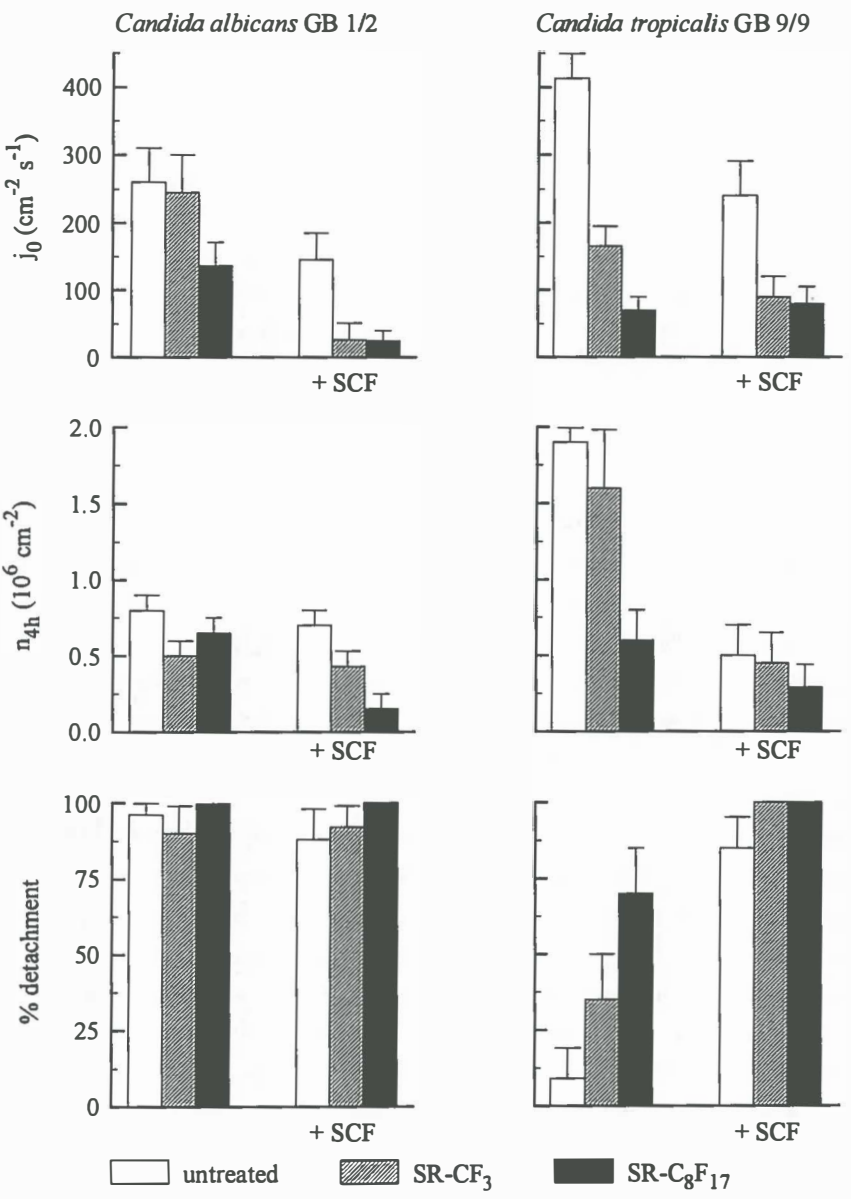


Figure 4. *Initial deposition rates j_0, numbers of adhering yeasts after 4 hours n_{4h}, and percentages of yeasts detached after passing an air-bubble through the flow chamber to Ar-SR-CF_3 and SR-C_8F_{17} surfaces and untreated silicone rubber, in the absence and presence of a salivary conditioning film (SCF), for two yeast strains; C. tropicalis GB 9/9 (left hand panel) and C. albicans GB 1/2 (right hand panel).*

water contact angles, Silver *et al.* (1995) could only increase the water contact angles of silicone rubber surfaces by 5 degrees from 108.5 to 113.6 degrees upon fluoro-alkyl chemisorption after oxygen plasma treatment. The water contact angles of the fluoro-alkylsiloxane layers measured in this study are much higher than of polytetrafluoroethylene or fluoroethylene-propylene surfaces, despite the similarity in surface chemistry. Kobayashi and Owen (1990) attributed this to the combination of the mobile, low surface free energy fluoro-alkyl chains with an entropic effect.

By consequence of the increased hydrophobicity and entropy of the silicone rubber surface after fluoro-alkylsilane chemisorption, the adhesion of bacteria and yeasts has significantly reduced. This reduction occurred both in the absence and presence of a salivary conditioning film on the substrata indicating that the salivary conditioning film transfered the properties of the underlying silicone rubber to the interface with adhering microorganisms, presumably through selective protein adsorption, displacement phenomena and conformational changes of the adsorbed proteins (Schakenraad *et al.*, 1987; Pratt-Terpstra *et al.*, 1988; 1989, van der Mei *et al.*, 1993). However, despite a significant reduction in microbial adhesion, the number of adhering organisms is not zero and growth may, albeit delayed, ultimately lead to the formation of a biofilm on the modified silicone rubber when used e.g. as a voice prostheses. More important than adhesion, is the ability of the initially adhering microorganisms to withstand occasionally high detachment forces. In the oropharyngeal cavity, a passing bolus or drinks may occasionally exert a high detachment force on the biofilm adhering through initial colonizers to silicone rubber voice prostheses. In the parallel plate flow chamber such high detachment forces can be created by passing an air-bubble through the chamber (Pitt *et al.*, 1993; Noordmans *et al.*, in press 1997). A very promising aspect of chemisorbed long chain fluoro-alkylsiloxanes to silicone rubber is that they not only reduce microbial adhesion but they also increase the percentage detachment by the passage of an air-bubble to almost 100%. Fig. 6 demonstrates that after the passage of an air-bubble through the parallel plate flow chamber, the number of microorganisms able to remain adhering is extremely low for all microorganisms, bacteria and yeasts, studied. The data in Fig. 6 may be interpreted to represent microbial adhesion under dynamic detachment conditions and may have a greater practical importance than the fundamentally interesting constant shear data presented in Figs. 3 and 4. This promising feature of adsorbed long chain fluoro-alkylsilanes is probably caused by a combination of the low surface free energy of the surface created in combination with the mobility of the adsorbed chain. Hydrophilic, mobile polyethylene glycol (PEG) chains, adsorbed to polystyrene surfaces, shows less protein adsorption (Holmberg *et al.*, 1993; Amiji and Park, 1993) and have reduced accumulation of dental plaque *in vivo* owing to the high entropy of the mobile chains.

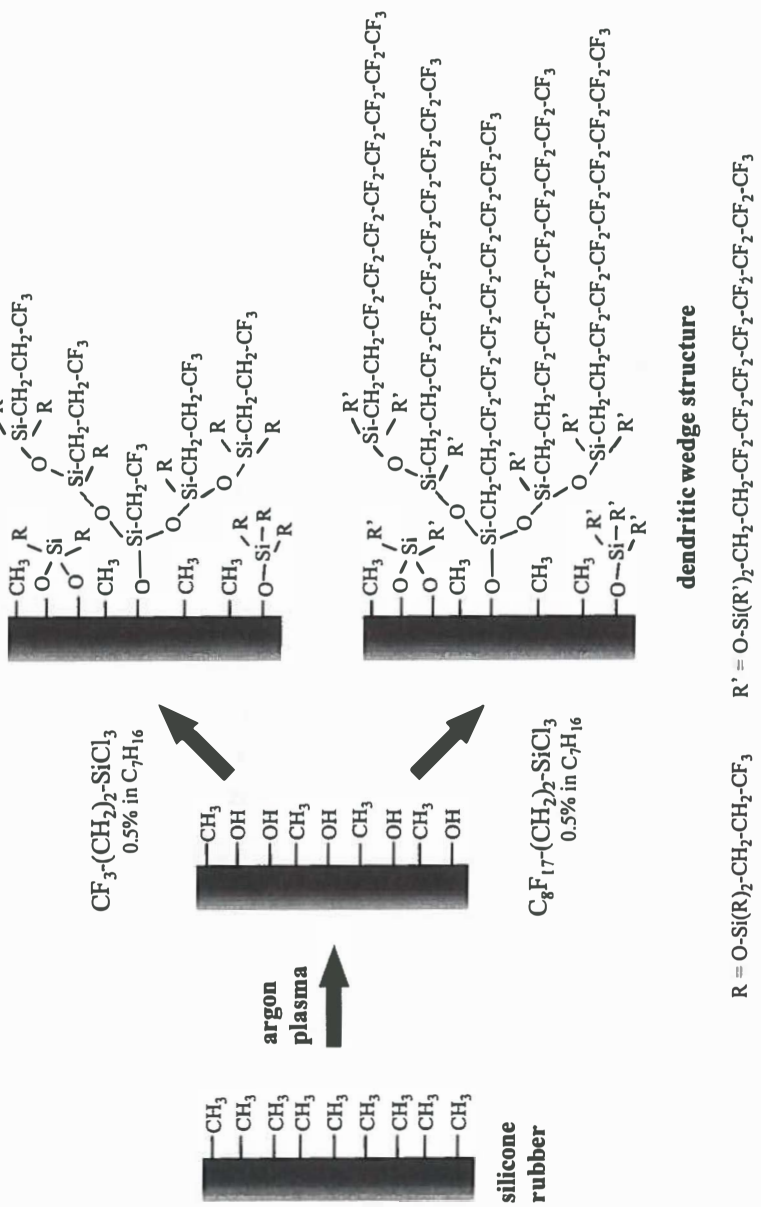


Figure 5. *Hypothetical presentation of fluoro-alkyl siloxane layers on argon plasma treated silicone rubber.*

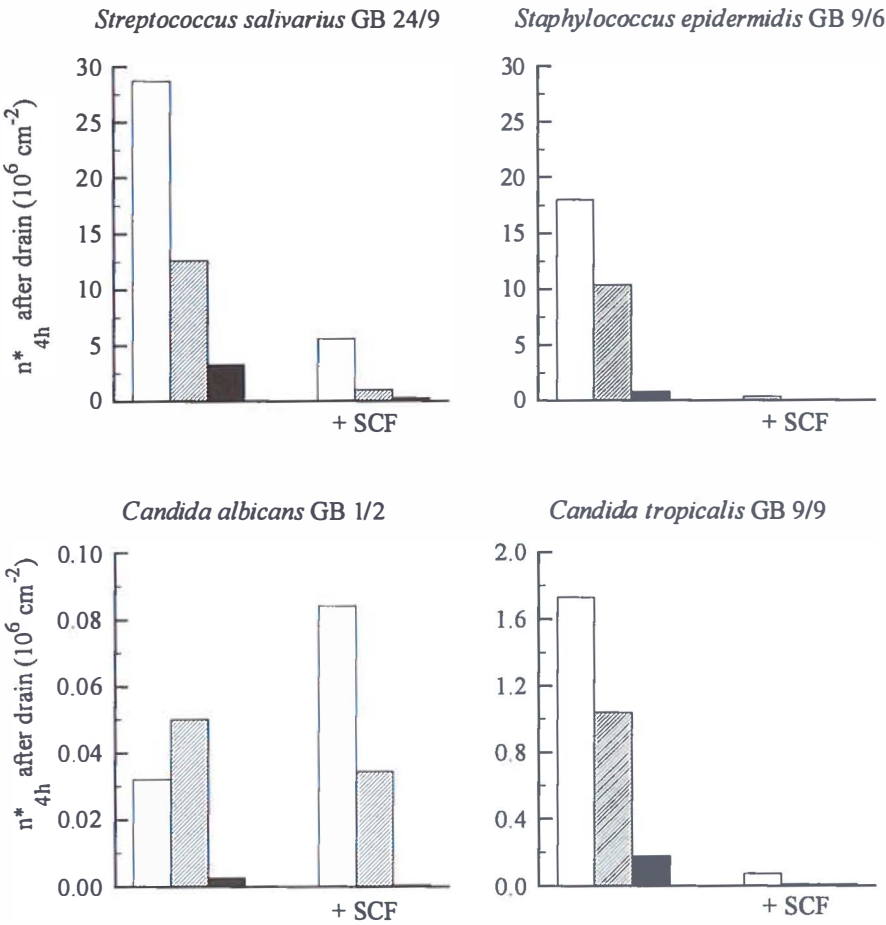


Figure 6. *Numbers of microorganisms (adhesion time 4 hours) n^*_{4h}, able to withstand the passage of an air-bubble through the flow chamber on Ar-SR-CF_3 and Ar-SR-C_xF_y- surfaces and untreated silicone rubber in the absence and presence of a salivary conditioning film (SCF).*

CONCLUSION

Silicone rubber can be made significantly more hydrophobic by chemisorption of short or long chain fluoro-alkyltrichlorosilanes after argon plasma treatment. The corresponding fluoro-alkylsiloxanes layers may yield a dendritic wedge structure with mobile chemisorbed chains. Adhesion of bacteria

CONCLUSION

Silicone rubber can be made significantly more hydrophobic by chemisorption of short or long chain fluoro-alkyltrichlorosilanes after argon plasma treatment. The corresponding fluoro-alkylsiloxanes layers may yield a dendritic wedge structure with mobile chemisorbed chains. Adhesion of bacteria and yeasts was significantly reduced to both chemisorbed short and long chain fluoro-alkylsiloxanes in the absence and presence of a salivary conditioning film. By comparison, Ar-SR-C_8F_{17} surfaces were less adhesive for bacteria and yeasts than Ar-SR-CF_3 surfaces. Moreover, detachment of adsorbed microorganisms was extremely easy on Ar-SR-C_8F_{17} surfaces, making it a promising candidate for the preparation of low fouling, silicone rubber voice prostheses.

REFERENCES

Absolom DR (1988), The role of bacterial hydrophobicity in infection, bacterial adhesion and phagoscytic ingestion. *Can. J. Microbiol.* **34**:287-299.

Amiji M and Park K (1993), Surface modification of polymeric biomaterials with poly(ethyleneoxide), albumin, and heparin for reduced thrombogenicity. *J. Biomater. Sci. Polymer Edn* **4**:217-234.

Beamson G and Briggs D (Eds) (1992), In: High resolution XPS of Organic Polymers. The Scienta ESCA 300 Database, John Wiley, Chichester, pp 268-269.

Busscher HJ, Bos R and van der Mei HC (1995), Initial microbial adhesion is a determinant for the strenght of biofilm adhesion. *FEMS Microbiology Letters* **128**:229-234.

Busscher HJ, de Boer CE, Verkerke GJ, Kalicharan R, Schutte HK and van der Mei HC (1994), In vitro ingrowth of yeasts into medical grade silicone rubber. *Int. Biodeterioration Biodegradation* **33**:383-390.

Busscher HJ and van der Mei HC (1995), Use of flow chamber devices and image analysis methods to study microbial adhesion. In: Methods in Enzymology, R.J. Doyle and I. Ofek (Eds), pp. 455-477. Academic Press, Inc., Florida.

Busscher HJ and Weerkamp AH (1987), Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiol. Rev.* **46**:165-173.

Everaert EPJM, Mahieu HF, Reitsma AM, van de Belt-Gritter B, van der Mei HC, Verkerke GJ, Dijk F and Busscher HJ (1996), Adhesion of yeasts and bacteria to fluoro-alkylsiloxane layers chemisorbed on silicone rubber. *J. Mat. Sci.:Mat. in Med.* submitted.

Everaert EPJM, Mahieu HF, Wong Chung RP, Verkerke GJ, van der Mei HC and Busscher HJ (1997), A new method for in vivo evaluation of biofilms on surface-modified silicone rubber voice prostheses. *Eur. Arch. Otorhinolaryngol.* **254**:261-263.

Everaert EP, van der Mei HC and Busscher HJ (1996), XPS analyses of plasma-treated silicone rubber. In: Surface Modification of Polymeric Biomaterials. B.D. Ratner and D.G. Castner (Eds), pp. 89-96. Plenum Press, New York.

Everaert EP, van der Mei HC, De Vries J and Busscher HJ (1995), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 1. Storage in air. *J. Adhesion Sci. Technol.* 9:1263-1278.

Kobayashi H and Owen MJ (1990), Surface tension of poly[(3,3,4,4,5,5,6,6,6,-nonafluorohexyl)-methylsiloxane] *Macromolecules* **23:**4929.

Leenaars AFM and O'Brien SBG (1989), Particle removal from silicon substrates using surface tension forces. *Philips J. Res.* **44:**183-209.

Mahieu HF, van Saene JJM and den Besten J (1986), Oropharynx decontamination preventing Candida vegetation on voice prostheses. *Arch. Otolaryngol. Head Neck Surg.* **112:**1090-1092.

Mozes N, Leonard AJ and Rouxhet PG (1988), On the relation between the elemental surface composition of yeasts and bacteria and their charge and hydrophobicity. *Biochim. et Biophys. Acta.* **945:**324-334.

Neu TR, van der Mei HC, Busscher HJ, Dijk F and Verkerke GJ (1993), Biodegradation of medical-grade silicone rubber used for voice prostheses: A sem STUDY. *Biomaterials* **14:**459-464.

Neu TR, Verkerke GJ, Herrmann IF, Schutte HK, van der Mei HC and Busscher HJ (1994), Microflora on explanted silicone rubber voice prostheses: taxonomy, hydrophobicity and electrophoretic mobility. *J. Appl. Bacteriology* **76:**521-528.

Noordmans J, Wit PJ, van der Mei HC and Busscher HJ (1997), Detachment of polystyrene particles from collector surface tension forces induced by air-bubble passage through a parallel plate flow chamber. *J. Adhesion Sci. Technol.* **11:**in press.

Olsson J, Carlén A and Holmberg K (1991), Inhibition of Streptococcus mutans adherence by means of surface treatment with combinations of alkylphosphates and nonionic surfactants. *Caries Res.* **25:**51-57.

Pitt WG, McBride MO, Barton AJ and Sagers RD (1993), Air-water interfaces displaces adsorbed bacteria. *Biomaterials* **14:**605-608.

Pratt-Terpstra IH, Weerkamp AH and Busscher HJ (1988), Microbial factors in a thermodynamic approach of oral streptococcal adhesion to solid substrata. *J. Colloid Interface Sci.* **129:**568-574.

Pratt-Terpstra IH, Weerkamp AH and Busscher HJ (1989), Microbial factors in a thermodynamic approach of oral streptococcal adhesion to human enamel and artificial substrata with various surface free energies. *J. Dent. Res.* **68:**463-467.

Quirynen M, Marechal M, van Steenberghe D, Busscher HJ and van der Mei HC (1991), The bacterial colonization of intra-oral hard surfaces in vivo: influence of surface free energy and surface roughness. *Biofouling* **4:**187-198.

Schakenraad JM, Kuit JH, Arends J, Busscher HJ, Feijen J and Wildevuur ChRH (1987), In vivo quantification of cell-polymer interactions. *Biomaterials* **8:**207-210.

Schneider RP and Marshall KC (1994), Retention of the Gram-negative marine bacterium SW8 on surfaces effects of microbial physiology, substratum nature and conditioning films. *Coll. Surf. B:Biointerfaces* **2:**387-396.

Silver JH, Hergenrother RW, Lin JC, Lim F, Lin HB, Okada T, Chaudhury MK and Cooper SL (1995), Surface and blood-contacting properties of alkylsiloxane monolayers supported on silicone rubber. *J. Biomed. Mat. Res.* **29:**535.

Van Dijk LJ, Goldsweer R and Busscher HJ (1988), Interfacial free energy as a driving force for pellicle formation in the oral cavity. *Biofouling* **1:**19-25.

Van den Hoogen FGA, Oudes MJ, Janssen P, Hombergen G, Nijdam HF and Manni JJ (1996), The Groningen, Nijdam and Provox voice prostheses: a prospective clinical comparrison based on 845 replacement. *Acta Otolaryngol.* (Stockh), **116**:119-124.

Van der Mei HC, Cox SD, Geertsema-Doornbusch GI, Doyle RJ and Busscher HJ (1993), A critical appraisal of positive cooperativity in oral streptococcal adhesion: Scatchard analyses of adhesion data versus analyses of the spacial arrangement of adhering bacteria. *J. Gen. Microbiol.* **139**:937-948.

REDUCED BIOFILM FORMATION *IN VIVO* ON PERFLUORO-ALKYLSILOXANE SURFACE MODIFIED GRONINGEN BUTTON VOICE PROSTHESES

E.P.J.M. Everaert, H.F. Mahieu, B. van de Belt-Gritter, A.M. Reitsma, G.J. Verkerke, H.C. van der Mei and H.J. Busscher*

ABSTRACT *Candida* colonization on indwelling, silicone rubber voice prostheses may cause an increase in airflow resistance and impairs the valve function, eventually resulting in replacement of the prosthesis. Microbial adhesion on biomedical implants, including colonization by *Candida* spp. of silicone rubber voice prostheses, is governed by properties of the biomaterials surface. In this paper, *in vivo* biofilm formation on perfluoro-alkylsiloxane modified silicone rubber voice prostheses is evaluated using partially surface modified Groningen button voice prostheses, of which only one side was chemically modified. Partially modified prostheses were placed in patients for a planned evaluation period of approximately 4 weeks. After removal, the surface area covered with biofilm on the oesophageal side was determined microscopically after which culture samples were taken from each side of a prosthesis and prostheses were prepared for electron microscopy. Both the planimetrical biofilm scores as well as the numbers of CFU's cm^{-2} were less on silicone rubber after chemisorption of long perfluoro-alkylsiloxane chains. Identical fungal strains, mainly *Candida* spp., were isolated from biofilms on each side of a oesophageal flange. However, the number of bacterial strains and species on the untreated, silicone rubber sides was notably larger than on the surface modified sides, with staphylococci being the most commonly isolated. It is concluded, that chemisorption of perfluoro-alkylsiloxanes to silicone rubber used for voice prostheses reduces biofilm formation *in vivo* and therefore likely prolongs the life-time of indwelling voice prostheses.

INTRODUCTION

Tracheo-esophageal (TE) puncture prostheses have become the most frequent method of post-laryngectomy voice rehabilitation (Mahieu, 1988; van Weissenbruch *et al.*, 1992; Lith-Bijl *et al.*, 1992). A major drawback of indwelling voice prostheses, however, involves colonization of the prostheses within several weeks by a thick biofilm that consists of a variety of adhering yeast and bacterial strains causing an increase in airflow resistance and impairing the valve function (Mahieu *et al.*, 1986a; Palmer *et al.*, 1993; Neu *et al.*, 1992; 1993). As a consequence, indwelling silicone rubber

*to be submitted to: Ann. Otol. Rhinol. Laryngol. (1997)

voice prostheses, such as the Groningen button, Provox or Nijdam prostheses have to be replaced on average every 4 months (van den Hoogen *et al.*, 1996; Hilgers and Balm, 1993). Analysis of the biofilms on voice prostheses removed from patients demonstrated that the colonizing yeast strains were often *Candida albicans* and *Candida tropicalis* (Mahieu *et al.*, 1986; Palmer *et al.*, 1993; Neu *et al.*, 1994a;b). Bacterial strains identified were of oral origin an included *Streptococcus mitis*, *Streptococcus sobrinus* and *Streptococcus salivarius* or were commensals from skin, such as *Staphylococcus epidermidis* and other staphylococcal isolates (Neu *et al.*, 1994a).

Various attempts have been made to retard biofilm formation on indwelling voice prostheses in efforts to prolong the device life-time, with varying degrees of success. These attempts include daily intake of large amounts of dairy products, such as Turkish yoghurt or Kephir containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains, use of a buccal bioadhesive slow-release tablet containing miconazole nitrate (van Weissenbruch *et al.*, 1997) as well as selective decontamination of the oropharyngeal cavity from yeast (Mahieu *et al.*, 1986b). Also, incorporation of up to 0.5-9 % (w/w) rifampicin in the silicone rubber has been tried by Schierholz (1997), but this affected the mechanical properties of the polymer.

Microbial adhesion to biomedical implants, including voice prostheses, is determined by the properties of the biomaterials surface, and an alternative method for preventing or retarding biofilm formation might involve modification of the silicone rubber surface. Recently, we have shown that the hydrophobicity of the silicone rubber surface influenced microbial adhesion to the silicone rubber *in vitro* (Everaert *et al.*, 1996; 1997a) and biofilm formation on voice prostheses *in vivo* even after four weeks use (Everaert *et al.*, 1997a,b). Further, also chemisorption of perfluoro-alkylsiloxanes had significantly reduced microbial adhesion to silicone rubber *in vitro* (Everaert *et al.*, 1997a). Moreover, detachment of adhering microorganisms was extremely easy from silicone rubber surfaces with chemisorbed, long chain perfluoro-alkylsiloxanes, making this modification promising for the preparation of low fouling, silicone rubber voice prostheses.

It is the aim of this paper to compare the biofilm formation on silicone rubber, Groningen button voice prostheses *in vivo*, with and without chemisorbed perfluoro-alkylsiloxanes.

MATERIALS AND METHODS

"Ultra-Low Resistance"Groningen button voice prostheses were kindly provided by Medin Instruments and Supplies (Groningen, The Netherlands). The silicone rubber was modified in a two-step process, as schematically shown in Fig. 1. Voice prostheses were first partially oxidized in an argon-plasma treatment (Everaert *et al.*, 1995). In a second step, perfluoro-alkyltrichlorosilanes (Fluka Chemie AG, Buchs, Switzerland) were chemisorbed onto the oxidized surfaces (10 min, 0.5 % in perfluoroheptane,

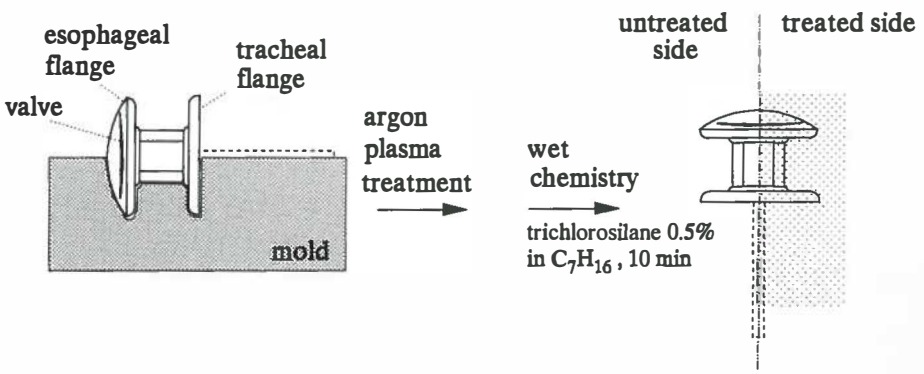


Figure 1. *Schematic illustration of the chemisorption of perfluoro-alkylsiloxanechains on one side of a silicone rubber voice prosthesis. During argon plasma treatment, one side of the prosthesis was shielded from the plasma by a plaster cast, while also only the argon plasma treated side was immersed in the silane solution.*

perfluoroheptane was chosen because this solvent does not swell the silicone rubber) by immersion of the oxidized part of the prostheses. Two different silanes with short (one perfluorocarbon unit) and long (eight perfluorocarbon units) perfluorocarbon chains were used. Silane-treated surfaces were washed with perfluoroheptane and absolute ethanol. Advancing water contact angles on silicone rubber with chemisorbed short and long perfluoro-alkylsiloxane chains were 125 ± 5 and 140 ± 5 degrees, respectively (Everaert *et al.*, 1997a), which is higher than on silicone rubber (advancing water contact angle 115 ± 3 degrees). Also, chemisorption of perfluoro-alkyl siloxane chains did not adversely affect the biocompatibility of the silicone rubber, according to an approved Agar diffusion test (BSC105/2, Bioscan BV, Bilthoven, The Netherlands). Partially modified Groningen button voice prostheses had a similar airflow resistance than the original prostheses (Rhinomanometer, Mercury NR3).

After being asked for consent, eight laryngectomized patients, having at least 6 months experience with the use of a voice prosthesis were given a partially modified Groningen button voice prosthesis for a planned period of approximately 4 weeks. Three patients received devices modified by chemisorption of short (one perfluorocarbon unit) chain perfluoro-alkylsilanes. Five patients received devices modified by chemisorption of long (eight perfluorocarbon units) chain perfluoro-alkylsilanes. After removal of the voice prosthesis from the tracheo-esophageal shunt, biofilm

was compared by light microscopy and a planimetric biofilm score was calculated as the percentage of the surface (of the esophageal flange) colonized by microorganisms. Subsequently, culture samples were taken and prostheses prepared for scanning electron microscopy. Microbial compositions of the biofilms on both sides of a valve were compared by plating on brain-heart infusion and blood agar plates at 37°C under aerobic conditions (Neu *et al.*, 1994a). Also, the number of colony forming units per unit area (CFU cm^{-2}) was determined for each side of a prosthesis as a second, quantitative measure for biofilm formation.

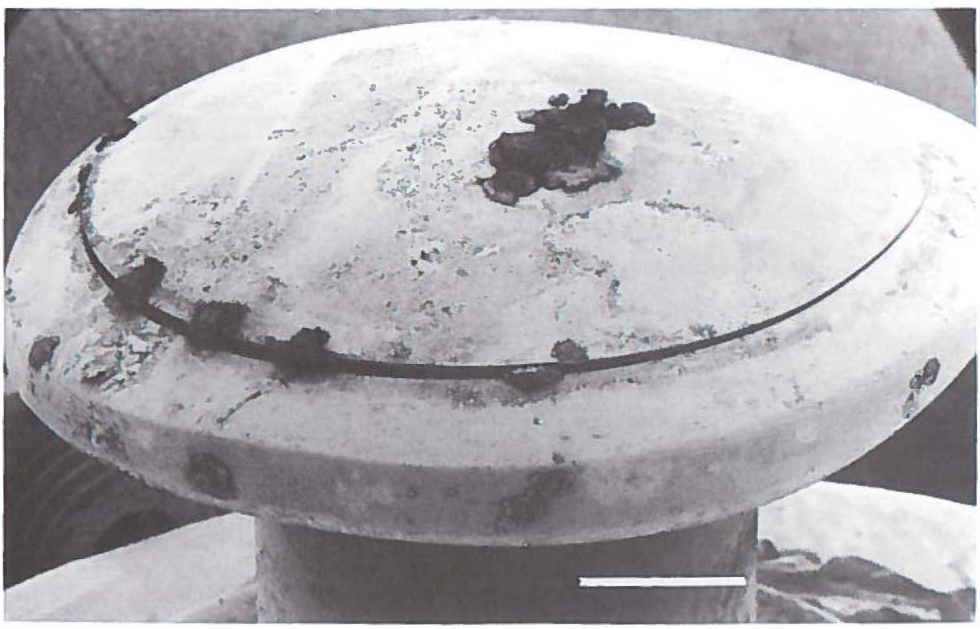


Figure 2. *Scanning electron micrographs of a partially surface modified, Groningen button voice prosthesis removed from a tracheo-esophageal shunt after four weeks of use. The right side of the prosthesis was modified by chemisorption of long perfluoro-alkylsiloxane chains (eight fluorocarbon units). The bar represents 2.0 mm.*

RESULTS

From the three patients who received devices modified by chemisorption of short chain perfluoro-alkylsilanes, it is noted that the surface modified sides attracted approximately double amounts of

biofilm than the original silicone rubber sides. On the basis of these results, we limited the *in vivo* evaluation of voice prostheses modified with short chain perfluoro-alkylsilanes to three patients.

In order to evaluate *in vivo* the effects of modifications having a surface combining higher surface entropy and hydrophobicity characteristics, five patients received devices modified by chemisorption of long chain perfluoro-alkylsilanes. Fig. 2 shows a scanning electron micrograph of the esophageal flange of a partially surface modified Groningen button voice prosthesis after four weeks use. On the modified side, long perfluoro-alkylsiloxane chains with eight perfluorocarbon units were chemisorbed to the silicone rubber. It can be seen that less microcolonies are formed on the surface modified side than on the original silicone rubber side of the prosthesis. Also, it is noted that the "big colony" centered on the SEM picture (Fig. 2) is placed just on the border between the modified and unmodified side of the device.

Table I summarizes the planimetric biofilm scores and the numbers of colony forming units per unit area on each side of the voice prostheses after chemisorption of the long chain perfluoroalkyl-silanes. Chemisorption of perfluoro-alkylsilanes with eight fluorocarbon units yielded a small reduction in planimetric biofilm scores but the accompanying reduction in CFU.cm^{-2} was significant and could become as high as tenfold.

Table I. The planimetric biofilm scores and numbers of colony forming units per unit area (CFU) on partially surface modified, Groningen button voice prostheses after chemisorption of long perfluoro-alkylsiloxane chains (eight fluorocarbon units). The prostheses were removed from the laryngectomized patients after a planned evaluation period of four weeks.

Patient	original side		perfluoro-alkylsiloxane side	
	planimetric biofilm score (%)	CFU (10^3.cm^{-2})	planimetric biofilm score (%)	CFU (10^3.cm^{-2})
M	5	168	5	74
N	10	460	2	46
Q	< 2	643	< 1	140
R				
S				

Note that results of patients R and S will be soon available.

In Table 2, the yeast and bacterial strains isolated from the biofilms on each side of a prosthesis are compiled. No shift in yeast composition of the biofilms occurred as a result of the surface modification by long chain perfluoroalkyl-silanes, with *C. albicans* being a commonly found strain. However, generally less different bacterial strains were isolated from the surface modified sides than from the original silicone rubber sides, although no new strains were found on the modified sides. Staphylococci were the most commonly isolated bacterial strains.

Table II. Microbial strains and species isolated from the original side (SR) and chemisorbed perfluoroalkylsiloxane side (with eight perfluorocarbon units, C_8F_{17}) of "Groningen Button" silicone rubber voice prostheses removed from laryngectomized patients, after a planned evaluation period of 4 weeks. "X" indicates detection of a single strain or species.

	Patient M		Patient N	
	SR	C_8F_{17}	SR	C_8F_{17}
Yeast strains				
Candida albicans	X	X	X	X
Candida glabrata			X	X
Candida krusei	X	X		
Bacterial strains				
CDC group E (act. spp)	X			
Genus pediococcus	X			
Staphylococcus aureus	X	X	X	X
non identified spp	XX	X	XX	

DISCUSSION

In this study we evaluated the possible potential of perfluoroalkyl-silane chemisorption on silicone rubber voice prostheses as a means to reduce biofilm formation and therewith prolong the prostheses life-time. The study is based on eight patients, which may at first glance appear inadequate to draw any conclusions. However, as previously described (Everaert *et al.*, 1997b), the use of partially surface

modified prostheses offers full control over all external factors influencing a comparison of biofilm formation on both sides including humidity, air temperature as well as nutrition factors and other variations in the patient's lifestyle. As consequence, the method allows conclusions to be drawn after a limited number of clinical trials. Moreover, the "split-button" method as employed here may well be the only method to evaluate biofilm formation on surface modified voice prostheses *in vivo*, simply because inter-patient variables are excluded.

The results of this study clearly indicate that chemisorption of long chain, perfluoroalkyl-silanes on silicone rubber yields reduced biofilm formation. The effects of the surface modification are most obvious from the number of CFU isolated per unit area than from the planimetric biofilm scores. This is because the planimetric biofilm score does not differentiate between thick and thin biofilms.

The reduction of biofilm formation on the long chain perfluoroalkyl-silane modified silicone rubber is a result of a variety of factors. First of all, the increased hydrophobicity of surface modified silicone rubber will contribute to the reduction in biofilm formation, as also demonstrated for dental plaque formation in the oral cavity (Burford-Mason *et al.*, 1988; Martin *et al.*, 1981). For the present application, increased hydrophobicity due to fluoridation of the silicone rubber alone is not sufficient as chemisorption of short chain, perfluoroalkyl-silanes do not show a favourable effect on biofilm formation. We have proposed that chemisorbed perfluoro-alkylsiloxane chains on silicone rubber form a dendritic wedge structure (Everaert *et al.*, 1997a) with a high degree of freedom of the chemisorbed chains. The favourable effects of chemisorbed long chain perfluoro-alkylsilanes as compared to short chain perfluoroalkyl-silanes can be attributed to a higher mobility of the chemisorbed chains, in a sense repelling the biofilm.

CONCLUSION

This study demonstrates that biofilm formation on silicone rubber Groningen button voice prostheses over a four weeks evaluation period can be reduced by chemisorption of long (eight perfluorocarbon units) perfluoro-alkylsiloxane polymer chains, owing to a combination of higher hydrophobicity and mobility of the chemisorbed polymer chains. Therewith, thus modified prostheses might be useful for those laryngectomees needing frequent replacement of their prosthesis. Whether or not the average life-time of indwelling voice prostheses in general of around 3-4 months can also be prolonged by the modification studied here remains to be determined by future research.

REFERENCES

Burford-Mason AP, Weber JCP, Willoughby JMT (1988), Oral carriage of Candida albicans, ABO blood group and secretor status in healthy subjects. *J. Med. Vet. Mycol.* **26**:49-56.

Everaert EPJM, van der Mei HC, de Vries J, Busscher HJ (1995), Hydrophobic recovery of repeatedly plasma-treated silicone rubber. Part 1. Storage in air. *J. Adhesion Sci. Technol.* **9**:1263-1278.

Everaert EPJM, Mahieu HF, Reitsma AM, van de Belt-Gritter B, van der Mei HC, Verkerke GJ, Dijk F, Busscher HJ (1996), In vitro and in vivo microbial adhesion and growth on argon plasma treated silicone rubber voice prostheses. *J. Mat. Sci.: Mat. in Med.* submitted

Everaert EPJM, van der Mei HC, Busscher HJ (1997a), Adhesion of yeasts and bacteria to fluoro-alkylsiloxanes layers chemisorbed on silicone rubber. *Colloids and Surfaces B: Biointerfaces.* submitted

Everaert EPJM, Mahieu HF, Wong Chung RP, Verkerke GJ, van der Mei HC, Busscher HJ (1997b), A new method for in vivo evaluation of biofilms on surface modified silicone rubber prostheses. *Eur. Arch. Otorhinolaryngol.* **254**:261-263.

Hilgers FJM, Balm AJM (1993), Long-term results of vocal rehabilitation after total laryngectomy with the low-resistance, indwelling Provox™ voice prosthesis system. *Clin. Otolaryngol.* **18**:517-523.

Lith-Bijl JT, Mahieu HF, Patel P, Zijlstra RJ (1992), Clinical experience with the low-resistance Groningen button. *Eur. Arch. Otorhinolaryngol.* **249**:354-357.

Mahieu HF, van Saene HKF, Rosingh HJ, Schutte HK (1986a), *Candida* vegetations on silicone voice prostheses. *Arch. Otolaryngol. Head Surg.* **112**:321-325.

Mahieu HF, van Saene JJM, Den Besten J, van Saene HKF (1986b), Oropharynx decontamination preventing *Candida* vegetation on voice prostheses. *Arch. Otolaryngol. Head Neck Surg.* **112**:1090-1092.

Mahieu HF (1988), Voice and Speech rehabilitation following laryngectomy. PhD Thesis, Groningen University, The Netherlands.

Martin MV, Al-Tikriti U, Bramley PA (1981), Yeast flora of the mouth and skin during and after irradiation for oral and laryngeal cancer. *J. Med. Microbiol.* **14**:457-467.

Neu TR, Dijk F, Verkerke GJ, van der Mei HC, Busscher HJ (1992), Scanning electron microscopy study of biofilms on silicone voice prostheses. *Cells and Materials* **2**:261-269.

Neu TR, van der Mei HC, Busscher HJ, Dijk F, Verkerke GJ (1993), Biodeterioration of medical-grade silicone rubber used for voice prostheses: a SEM study. *Biomaterials* **14**:459-464.

Neu TR, Verkerke GJ, Herrmann IF, Schutte HK, van der Mei HC, Busscher HJ (1994a), Microflora on explanted silicone rubber voice prostheses: taxonomy, hydrophobicity and electrophoretic mobility. *J. Appl. Bacteriol.* **76**:521-528.

Neu TR, de Boer CE, Verkerke GJ, Schutte HK, Rakhorst G, van der Mei HC, Busscher HJ (1994b), Biofilm development in time on a silicone voice prosthesis - A case study. *Microbial ecology in health and disease.* **7**:27-33.

Palmer MD, Johnson AP, Elliott TSJ (1993), Microbial colonization of Blom-Singer Prostheses in Postlaryngectomy Patients. *Laryngoscope* **103**:910-914.

Schierholz JM (1997), Physico-chemical properties of a rifampicin-releasing polydimethylsiloxane shunt. *Biomaterials* **18**:635-641.

Van den Hoogen FJA, Oudes MJ, Janssen P, Hombergen G, Nijdam HF, Manni JJ (1996), The Groningen, Nijdam and Provox Voice Prostheses: a prospective clinical comparison based on 845 replacement. *Acta. Otolaryngol. (Stockh)* **116**:119-124.

Van Weissenbruch R and Albers FWJ (1992), Voice rehabilitation after total laryngectomy. *Acta Oto-Rhino-Laryngol. Belg.* **46**:221-246.

Van Weissenbruch R (1996), Voice restoration after total laryngectomy. PhD Thesis, Groningen University, The Netherlands.

Van Weissenbruch R, Bouckaert S, Remon J-P, Nelis HJ, Aerts R, Albers FWJ (1996) Chemoprophylaxis of fungal deterioration of the Provox prosthesis. *Ann. Otol. Rhinol. Laryngol.*

GENERAL DISCUSSION

Synthetic polymers are used in millions of humans as medical applications each year again (Ratner, 1996). These polymer containing medical devices can be designed and synthetized to have specific mechanical properties, durability, and functionality, where the bulk structure of the materials governs these properties. Biological responses to biomaterials are, however, essentially dominated by their surface chemistry and structure. Consequently, proper use of surface modification allows the bulk mechanical properties and functionality of medical devices to remain unchanged with improved biological performance. However, a major limitation, commonly observed, of surface modifications is surface rearrangement. The hydrophobic recovery occurring on polymer surfaces freshly hydrophilized by plasma treatment is one example illustrating this problem. Surface chemistries and structures can drift from the "as formed" state due to rotation, translation or diffusion of surface atoms or molecules in response to the external environment. The driving forces for these surface changes are the minimization of interfacial energy and increasing entropy of the system. The modified region, concentrated at the polymer surface, will distribute itself uniformly throughout the bulk if sufficient molecular mobility exists. Thus, it is necessary to prevent rearrangements of e.g. plasma treated silicone rubber surfaces. Repeating the plasma treatment after partial recovery and subsequently storage in either water or liquid nitrogen has been suggested to hydrophilize silicone rubber surfaces on a more permanent basis (see chapters 2 and 3).

Speech is such an essential part of communication in normal human life, that it is almost impossible to imagine lacking the ability to communicate. Laryngectomees, amongst others, are patients who have to face a serious handicap to their ability to communicate. A laryngectomee once told me: "*The moment I knew that I had a cancer and I had to be laryngectomized, I though that my life no longer had any value. Communicating with people was very important to me, now this was being taken from me... Fortunately, I received the Groningen button allowing me the capability of speech and nowadays I can continue to communicate in an effective way. I overcame this handicap.*"

An essential drawback of silicone rubber voice prostheses, is engendered by microbial colonization. To date, prevention of microbial colonization of the devices is achieved using antifungal medication, either via oral administration (Mahieu *et al.*, 1986) or via buccal bioadhesives slow-release tablet containing miconazole (van Weissenbruch *et al.*, 1996). However, long term medication use is not a definitive solution with resistance to azoles having been shown for *C. albicans* and *C. glabrata*. Observed most commonly as mucosal candidosis in AIDS patients, with increased oropharyngeal or esophageal candidosis, necessitating further antifungal treatments (Denning, 1995). Moreover, once infection of polymer devices becomes well established, antimicrobial therapy is usually futile and the only effective course of action is removal of the device. Since biofilm formation on voice prostheses is, *in vivo*, governed by an interplay of a multi-factorial system involving the surface properties of the

device and its environment. Alternatives to antibiotics to prevent device colonization could possibly involve modifications of the device surface properties. Compellingly, a laryngectomee once said: "... *I find your method ideal because, in this case, I would have the possibility to forget that I have a voice prosthesis. ...the 3-4 times daily intake of antifungal tablets is, for me, annoying and not healthy.*"

Improvements to the anti-fouling properties of the voice prostheses material have been suggested in this thesis along with other important suspected factors to help in prolonging device lifetime. The silicone rubber voice prosthesis surface should possess the following properties in order to minimize microbial colonization: a combination of a higher entropy and hydrophobicity, a very smooth surface, a surface exempt of monomer content and an appropriately designed topography.

- Modified surface of silicone rubber voice prostheses owning a higher surface entropy and hydrophobicity showed improved anti-fouling properties as described in chapters 7 and 8. Surface modifications were obtained by the chemisorption of perfluoro-alkylsiloxane polymer chains on the silicone rubber devices (see chapter 7).
- Dependent upon the application, medical devices may require specific surface characteristics e.g., voice prostheses should possess a very smooth surface rather than porous, as needed for subcutaneous implants. The porous subcutaneous implant allows soft tissue and bone ingrowth into the implant providing its stability and by consequence, may behave more like host tissue (Sclafani *et al.*, 1997). In contrast, ingrowth of microorganisms into prostheses has to be inhibited as it impairs valve functions. The roughness of the silicone rubber voice prostheses may be decreased upon removing the tiny air nuclei often found in the surface by washing the devices in absolute ethanol for at least 24 h.
- It is suspected that microbial ingrowth into the silicone rubber devices is controlled by not only physical but also chemical mechanisms. The residual siloxane monomer always found in the Groningen button, may promote the chemical attack of the device by microorganisms. A post-curing (180 °C, 3 h) of the voice prostheses will complete the crosslinking of the silicone matrix and evaporates residual low molecular weight molecules.
- In the oropharyngeal cavity, high shear rates are created when patients swallow any liquid or nutrient. Higher shear rates diminish the ability of microorganisms to adhere to the substrate surface. Consequently, under dynamic flow, an appropriately designed topography of the device may become important in the adhesion process. Thus, regardless of how successfully a surface modification renders a biomaterial resistant to microbial adhesion, the design of a device may still render a device susceptible to microbial adhesion and subsequent invasion.
- Interestingly, it has been suggested for several years that the consumption of fermented dairy products by laryngectomees, such as buttermilk or Turkish yoghurt, prolongs the lifetime of

indwelling silicone rubber voice prostheses. Although a rigorous scientific basis for these statements is still lacking, ENT specialists should continue to encourage their patients to consume fermented dairy products.

In conclusion, we believe that the combination of the proposals in the thesis will improve the anti-fouling properties of silicone rubber voice prostheses. As a consequence, the lifetime of voice prostheses may be lengthened which would directly benefit laryngectomized patients. However, laryngectomees should keep in mind that the use of medical devices do not cure diseases, rather they correct the functional consequences of disease.

REFERENCES

Denning DW (1995), Can we prevent azole resistance in fungi?. *Lancet*, **346**:454-455.

Mahieu HF, van Saene JJM and den Besten J (1986), Oropharynx decontamination preventing Candida vegetation on voice prostheses. *Arch. Otholaryngol. Head Neck Surg.* **112**:1090-1092.

Ratner BD (1996), Surface modification of polymer for biomedical application: chemical, biological, and surface analytical challenges. In: Surface modification of polymeric biomaterials, Ratner BD and Castner DG (Eds), Plenum Press, New York, pp 1-9.

Sclafani AP, Thomas JR, Cox AJ and Cooper MH (1997), Clinical and histologic response of subcutaneous explanted polytetrafluoroethylene (Gore-Tex) and porous high-density polyethylene (Medpor) implant to acute and early infection. *Arch. Otolaryngol. head neck surg.* **123**:328-336.

Van Weissenbruch R, Bouckaert S, Remon JP, Nelis HJ, Aerts R and Albers FWJ (1997), Chemoprophylaxis of fungal deterioration of the Provox™ tracheoesophageal voice prosthesis in post-laryngectomy patients. *Ann. Otol. Laryngol.* **106**:329-337.

SUMMARY

Total laryngectomy is a surgical treatment for extensive cancer of the larynx or hypopharynx, which alters speech, swallowing, and respiration. During the last two decades, surgical voice restoration procedures comprising tracheo-esophageal puncture techniques with insertion of a so-called "voice prosthesis" have greatly improved successful voice acquisition following laryngectomy (see **Chapter 1** for a literature survey). Most voice prostheses are made of silicone rubber because of its excellent mechanical and molding properties. However, the major drawback is engendered by concurrent microbial colonization of the devices. Clinically, voice prostheses have to be replaced when, due to biofilm formation, patients complain about leakage of food and liquid or increased air flow resistance.

Infection and deterioration of implanted polymeric medical devices is a major problem in medicine. Since microbial adhesion to implanted devices is determined by its surface properties, prevention of biofilm formation could involve the modification of the surface properties of the voice prosthesis. As silicone rubber is known to be hydrophobic, we first attempted to hydrophilize the silicone rubber surface in order to enhance its anti-fouling properties. RF plasma treatment is often a useful method to hydrophilize polymer surfaces, but effects of a single plasma treatment of silicone rubber are usually transient due to the high mobility of its siloxane backbone. Therefore, in Chapter 2 and 3, solutions has been proposed to overcome this problem so-called hydrophobic recovery.

In **Chapter 2**, the physico-chemical surface properties of medical grade silicone rubber repeatedly modified by means of oxygen, argon, carbon dioxide, and ammonia RF plasma treatments, are characterized. Treated samples were stored in air prior to surface characterisation using water contact angle measurements, X-ray photoelectron spectroscopy, streaming potential measurements and profilometry for surface roughness. Argon, carbon dioxide and ammonia plasmas significantly reduced the advancing water contact angle from 115 to 58, 72, and 85 degrees, respectively on a more permanent basis (especially when the treatments were repeated after recovery). Oxygen plasma effects on water contact angles generally disappeared within 5 hours, also after repeated treatment. The formation of Si-O-Si bridges between siloxane chains after plasma-treatment was demonstrated by the appearance of a new component in the Si_{2p} peak but the degree to which this occurred differed per gas. Streaming potential measurements indicated a more negatively charged surface for treated samples compared to untreated samples. Surface roughness increased slightly for repeatedly plasma treated samples from R_A= 0.35 μm to 0.46 μm while scanning electron microscopy showed the presence of several "cracks" spanning the surface after repeated treatment.

In **Chapter 3**, the hydrophobic recovery of repeated plasma treatments on silicone rubber surfaces occurring in air, water, or liquid nitrogen, is compared. The temporal behavior of the effects of the plasma treatment on the physico-chemical surface properties of the silicone rubber were investigated using water contact angle measurements and X-ray photoelectron spectroscopy (XPS).

Hydrophobic recovery during 3 months storage in ambient air was considerable and nearly complete for all four plasmas used. Hydrophobic recovery was almost completely suppressed during storage in liquid nitrogen, and only a minor increase of around 10° in advancing water contact angle was observed for all four plasma treatments. Also during storage of treated samples in water, hydrophobic recovery was minimal and initiated again by returning the treated samples to ambient air. XPS analyses showed that argon, carbon dioxide and ammonia plasma treated silicone rubber all had increased carbon percentages at the expense of oxygen and silicon after storage in water, or in liquid nitrogen, as compared to after storage in ambient air. Interestingly, the carbon content of oxygen plasma treated silicone rubber decreased during storage in water, or in liquid nitrogen as compared to during storage in ambient air, while its oxygen and silicon percentages increased.

In **Chapter 4**, the surface restructuring in ambient air of medical grade silicone rubber surfaces modified by repeated RF plasma treatments using various discharge gases including oxygen, argon, carbon dioxide and ammonia, was studied quantitatively. From advancing and receding water contact angle data (see chapter 2), the fraction of the surface covered by mobile and immobile polar groups, and a characteristic time constant of the restructuring process were calculated. For argon plasma-treated surfaces, the fraction of immobile polar groups increased with repeated plasma treatments, but remained relatively constant for samples repeatedly treated by an ammonia plasma. The use of an oxygen plasma only yielded incorporation of mobile polar groups but not of immobile polar groups. The increase in the restructuring time constants of argon and ammonia plasma treated silicone rubber with the number of plasma treatments suggested enhanced crosslinking of the silicone rubber by these plasmas. In contrast, when an oxygen plasma was repeatedly used, the restructuring time constant decreased suggesting chain cleavage by an oxygen plasma.

Chapter 5 presents the effects of hydrophilized silicone rubber surfaces on *in vitro* adhesion and growth of bacteria and yeasts isolated from voice prostheses, as well as on the *in vivo* biofilm formation. It was demonstrated that *in vitro* microbial adhesion and growth on silicone rubber can be reduced upon argon plasma treatment. However, *in vivo* biofilm formation on silicone rubber voice prostheses is oppositely enhanced by hydrophilizing the silicone rubber surface. From the results of this study, we concluded that the hydrophobicity of the biomaterials surface used, is an important factor controlling *in vivo* biofilm formation on voice prostheses.

Chapter 6 presents a new method that permits a rapid and accurate longitudinal *in vivo* evaluation of biofilm formation on surface modified silicone rubber voice prostheses. The method is based on partial modification of a Groningen button voice prosthesis by exposing half of the prosthesis to an argon plasma, and denoted as "split-button" method. This resulted in one side of the prosthesis becoming hydrophilic while leaving the unmodified side hydrophobic as a control. Findings showed that the borderline between the modified and unmodified sides was clearly visible from the differential

fouling with enhanced biofilm formation on the hydrophilic side as compared to the hydrophobic side. The method of partial surface modification used was seen to be extremely suitable for demonstrating such influences regardless of nutrition and other variations in patient's lifestyle. Microbiological analysis of the biofilms on both sides of the prosthesis valve did not show any changes in microbial composition with *Candida albicans*, streptococci and staphylococci being the most commonly isolated strains.

Whereas, we reported in chapter 6 that biofilm formation was, *in vivo*, enhanced on hydrophilized silicone rubber surfaces, we endeavoured to synthesize new surface properties of the devices combining high hydrophobicity and surface entropy. In **Chapter 7**, we report on the *in vitro* adhesion of yeasts and bacteria to silicone rubber surfaces made highly hydrophobic, upon chemisorption of either short or long fluoro-alkylsiloxane polymer chains. Physico-chemical properties of the chemisorbed layers were studied by using water contact angle measurements, X-ray photoelectron spectroscopy (XPS) and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Using a parallel plate flow chamber, adhesion of *Streptococcus salivarius*, *Staphylococcus epidermidis*, *Candida albicans* and *Candida tropicalis* strains, isolated from explanted voice prostheses, was investigated to the chemisorbed fluoro-alkylsiloxane layers with and without a salivary conditioning film. Treated surfaces showed significantly reduced microbial adhesion as compared to original silicone rubber, both with respect to initial deposition rates as well as with respect to adhesion in a stationary end-point. Furthermore, adhering microorganisms were more easily detached when applying an air-liquid interface. Silicone rubber surfaces with chemisorbed, long fluorocarbon chains showed the greatest reduction in microbial adhesion, probably because of their low surface free energy combined with a higher surface entropy.

In **Chapter 8**, *in vivo* evaluation of biofilm formation on silicone rubber voice prostheses made highly hydrophobic by chemisorption of long perfluoro-alkylsiloxane polymer chains will be presented. The method used was based on partial modification of a Groningen button voice prosthesis resulting in one side of the prosthesis becoming highly hydrophobic while leaving the unmodified side as a control. Findings showed that less biofilm formation occurred on the voice prostheses side made highly hydrophobic as compared to the unmodified side of the device. Consequently, chemisorption of long perfluoro-alkylsiloxane polymer chains on silicone rubber surface, may lengthen the voice prostheses lifetime. Microbiological analysis of the biofilms on both sides of the esophageal flange of the prosthesis revealed that the yeast composition was largely the same on either side, with *Candida* spp mostly isolated. For the bacteria, we found that the number of different species was notably larger on the untreated side, with staphylococci being the most commonly isolated strains.

In **Chapter 9**, the general discussion to this thesis, suggestions are made to improve the anti-fouling properties of silicone rubber voice prostheses.

Summarizing, this thesis demonstrates that the "split-button" method as employed in this thesis may well be the only method to evaluate biofilm formation on surface modified voice prostheses *in vivo*. Further, upon appropriate surface treatments of silicone rubber voice prostheses, concurrent colonization of the device could be reduced. As a consequence, the lifetime of voice prostheses may, most probably, be lengthened which would directly benefit laryngectomized patients.

SAMENVATTING

Kanker is altijd een ziekte die diep ingrijpt in het leven van de patient en dit geldt in het bijzonder wanneer het strottehoofd erdoor wordt getroffen. Hier immers bevinden zich de stembanden, die de spraak mogelijk maken. Een tumor in dit gebied betekent doorgaans dat het gehele strottehoofd operatief moet worden verwijderd, de zogenaamde totale laryngectomie. In eerste instantie verliest de patient hierdoor zijn spraakvermogen. Wat dit voor een mens betekent kan misschien het best worden geïllustreerd door middel van de uitspraak van een door keelkanker getroffen man: "*Toen ik wist dat ik keelkanker had en gelaryngectomeerd moest worden was het leven voor mij niets meer waard. Ik zou niet meer kunnen communiceren zoals ik gewend was. Het was een regelrechte ramp.*"

Gelukkig is er wel iets te doen tegen het bovengenoemde spraakverlies. De spieren die na de laryngectomie in de keelholte zijn blijven zitten zijn meestal in staat om de funktie van de weggenomen stembanden over te nemen. Om te kunnen spreken heeft men echter nog wel een luchtbron nodig die de spieren in trilling kan brengen. Aanvankelijk moest de patient hiervoor lucht inslikken, om die via de slokdarm weer "uit te boeren". In de praktijk verloopt dit moeizaam omdat de slokdarm slechts een kleine hoeveelheid lucht kan bevatten. In de woorden van de hierboven geciteerde patient was slokdarmspraak " (...) *een grote teleurstelling. Ik kon niet meer communiceren.*"

Al snel is daarom geprobeerd om het veel grotere volume van de longen te benutten om de benodigde luchtstroom voort te brengen. Hiervoor is het echter nodig om een opening te maken tussen luchtpijp en slokdarm. Het strottehoofd, dat normaal gesproken een soort kruispunt vormt waar luchtstroom en voedselweg elkaar passeren, is immers niet meer aanwezig. De kunstmatige opening moet ook afgesloten kunnen worden als de patient eet of drinkt, om te voorkomen dat er voedsel in de luchtpijp terecht komt. Dit kan door een soort ventiel in de opening te plaatsen. Dit ventiel wordt shunt-ventiel of ook wel stemprothese genoemd.

De Amerikaan E. D. Blom was de eerste die een goed werkende stemprothese ontwikkelde. Zijn zogenoemde "Eendensnavel" kwam in 1979 op de markt, maar is nu grotendeels overvleugeld door enkele andere typen stemprothesen, waaronder ook het in Groningen ontwikkelde "Groninger knoopje". Dat deze prothese goed werkt moge blijken uit het relaas van bovengenoemde patient die erover opmerkte: "*Gelukkig heb ik het Groninger knoopje gekregen. Ik ben nu niet meer gehandicapt en kan weer effectief communiceren*".

Alles lijkt dus dik voor elkaar wat betreft de stemprothese, maar helaas is de werkelijkheid anders. Dat komt door het materiaal waar de prothese van is gemaakt. Het enige materiaal dat hiervoor voldoet is siliconenrubber, dit is namelijk erg soepel, niet giftig en gemakkelijk in allerlei vormen te vervaardigen. Het enige nadeel is dat het binnen de kortste keren overwoekerd raakt door de in de mondholte levende bacteriën, schimmels en gistcellen. De prothese gaat hierdoor lekken, waardoor de patient het gevoel krijgt dat hij zich verslikt, en de luchtweerstand neemt toe, wat het spreken sterk

bemoeilijkt. Gemiddeld moet een prothese daarom om de drie à vier maanden worden vervangen, hetgeen door de patient als een vervelende ingreep wordt ervaren. Veel patiënten slikken daarom 3 à 4 keer per dag een anti-gist pil, maar dat is voor de patiënt, zeker op lange termijn, ook niet bepaald gezond.

Vandaar dat er hard wordt gestudeerd op manieren om de ingroei van micro-organismen in de prothese althans te vertragen. Omdat begroeiïng aan het oppervlak plaats vindt, ligt het voor de hand om te proberen iets aan het oppervlak van de prothese te veranderen. Nu is al heel lang bekend dat verschillende micro-organismen een voorkeur hebben voor verschillende soorten oppervlakken. In het bijzonder speelt het een belangrijke rol of het oppervlak hydrofoob of hydrofiel is. Hydrofobe oppervlakken stoten water af. Een voorbeeld hiervan is het welbekende Tefal-pannetje. Hierop blijft het water in dikke druppels liggen. De contacthoek tussen het wateroppervlak en het oppervlak van het pannetje is groot. Hydrofiele oppervlakken, zoals een schone glasplaat, werken juist aantrekkend op water. De contacthoek tussen het water en het glas is klein. Siliconenrubber is van nature sterk hydrofoob. Het lag dus voor de hand om te proberen de ingroei van micro-organismen te verminderen door dit materiaal hydrofiel te maken. Dit kan onder meer door het siliconenrubber met een gas-plasma te behandelen.

Om erachter te komen welk gas in de praktijk het beste werkt, hebben we het rubber met verschillende plasma-gassen (zuurstof, argon, kooldioxide en ammoniak) behandeld. De fysisch-chemische eigenschappen van de behandelde oppervlakken hebben we gekarakteriseerd met een reeks technieken, zoals randhoekmeting, Electron Spectroscopy for Chemical Analysis (ESCA), meting van de stromingspotentiaal, scanning electronen microscopie en oppervlakteruwheid. Het bleek al snel dat de hydrofiliciteit die door de plasmabehandeling werd verkregen, niet blijvend was, het zogenoemde hydrofobe herstel. Dit wordt veroorzaakt door de hoge mobiliteit van de moleculaire siloxaanketens waaruit het siliconenrubber is opgebouwd. We vonden echter dat de winst in hydrofiliciteit sterk te verbeteren viel door argon als plasma-gas te gebruiken en het siliconenrubber niet één maar meerdere malen te behandelen, met tussenpozen van 24 uur (**hoofdstuk 2**). Bovendien bleek het hydrophobe herstel na behandeling vrijwel te stoppen door het behandelde siliconenrubber te bewaren in steriel water (**hoofdstuk 3**).

In een poging het mechanisme dat aan het hydrofobe herstel ten grondslag ligt enigzins kwantitatief te doorgronden, hebben we een model toegepast dat was ontwikkeld door dr. R. C. Chatelier uit Melbourne, Australië (**hoofdstuk 4**). In theorie zijn het de zogenaamde polaire groepen die verantwoordelijk zijn voor het hydrofiele karakter van het oppervlak. Het hydrofobe herstel vindt plaats doordat deze polaire groepen gedeeltelijk mobiel zijn en zich na verloop van tijd van het oppervlak af richten. Uitgaande van metingen van de contacthoek geeft dit model ons de fractie van het oppervlak dat wordt ingenomen door mobiele dan wel immobiele polaire groepen en de

tijdconstante die de reöriëntatie van de mobiele groepen bepaalt. We vonden nu dat zowel de mobiele fractie als de tijdconstante veranderden als funktie van het aantal behandelingen. Dit gebeurde voor elk van de vier gebruikte plasma-gassen. De veranderingen bleken goed te kunnen worden verklaard door aan te nemen dat plasmabehandeling met argon en ammoniak de mate van cross-linking aan het oppervlak verhoogt, terwijl behandeling met zuurstof en kooldioxide deze juist verlaagt.

Hoofdstuk 5 beschrijft de *in vitro* tests die zijn uitgevoerd om erachter te komen hoe de hydrofiel gemaakte prothese zich gedraagt. We hebben dit getest in zowel de flow cell als in het zogenaamde Robbins device. In de eerste wordt een suspensie van geselecteerde micro-organismen gedurende vier uur bij kamertemperatuur langs het oppervlak geleid. Het hechtingsgedrag wordt gelijktijdig bestudeerd door een microscoop. Het Robbins device werkt vergelijkbaar, alleen is de temperatuur van de suspensie 37 °C, kan er voedsel aan de suspensie worden toegevoegd, en is de duur van het experiment twee weken. Het Robbins device geeft dus in het algemeen een betere weergave van de werkelijkheid. De uitkomst was dat van de vier soorten geselecteerde micro-organismen (twee bacteriën en twee gisten) er één zich even sterk aan het hydrofiel gemaakte oppervlak hechtte als aan het onbehandelde oppervlak en drie duidelijk minder sterk.

Deze uitkomst was dermate hoopgevend dat hij een *in vivo* experiment rechtvaardigde. Maar voor dit kon gebeuren moesten we eerst nagaan of de behandelde prothese in mechanische en toxische eigenschappen niet onderdeed voor een onbehandelde. Gelukkig bleek dit inderdaad het geval en kon het *in vivo* experiment doorgaan. Een probleem hierbij echter lag in het feit dat de snelheid waarmee de prothese begroeid raakt van patiënt tot patiënt enorm uiteen kan lopen en zelfs in dezelfde patiënt lang niet altijd hetzelfde is. Het is dus lastig om de hechtingseigenschappen van een behandelde prothese te vergelijken met die van een onbehandelde, tenzij men beschikt over een zo grote groep patiënten dat op de uitkomst van de studie statistiek kan worden bedreven. Dit was is ons geval niet zo en dus moesten we wat anders verzinnen.

Onze oplossing bestond eruit dat we de prothese tijdens de plasma-behandeling voor de helft in een gipsen mal zetten. Hierdoor werd de helft van het prothese-oppervlak van de buitenwereld afgesloten en bleef dus onbehandeld (**hoofdstuk 6**). Dit was bijzonder mooi te zien aan een prothese die, na aldus behandeld te zijn in water werd ondergedompeld. Aan de hydrofiel gemaakte kant bleef het water hangen, aan de onbehandelde, hydrofobe, kant liep het er meteen af. De scheiding tussen beide kanten was messcherp en kaarsrecht. Door deze kunstgreep, de split-button techniek in vakjargon, konden alle verschillen die toe te schrijven zouden kunnen zijn aan verschillen in mondflora en levensstijl tussen patiënten worden geëlimineerd.

Alles liep dus prachtig en niets leek een succesvolle *in vivo* studie meer in de weg te staan, maar helaas moesten we ervaren dat het onderzoekersbestaan niet altijd over rozen gaat. Op de eerste

prothese die, na een maand, uit een patiënt werd gehaald was nog steeds een messcherpe en kaarsrechte scheiding te zien, dat wel, maar wel met de meeste begroeiing op de behandelde, hydrophiele, kant. De zes andere prothesen, die later werden geëxplanteerd, gaven hetzelfde beeld te zien. Wat soorten micro-organismen betreft zat er niet veel verschil tussen beide kanten (**hoofdstukken 5 en 6**). Kennelijk is de hydrophobiciteit van het oppervlak een belangrijke parameter voor microbiële hechting, maar gedragen de micro-organismen in de mondholte zich wezenlijk anders dan die in een laboratoriumopstelling.

Omdat hydrophiel maken van een stemprothese niet werkt, maar hydrophobiciteit wel een belangrijke rol speelt, lag het in zekere zin voor de hand om te proberen het oppervlak juist nog hydrophober te maken. Het bovengenoemde Tefal-pannetje ontleent zijn roemruchte bakkwaliteiten aan een laagje zogenaamd teflon. Dit bestaat uit lange koolstofketens waaraan fluoratomen gekoppeld zijn. Zou het mogelijk zijn om een teflon-achtig laagje op siliconenrubber aan te brengen, zo was dus de vraag. Uit mijn chemische achtergrond wist ik dat de zogenoemde chlorosilaanmoleculen (-SiCl) goed reactief koppelen met een hydroxylgroep (-OH). Hydroxylgroepen zijn op het siliconenrubber aan te brengen door plasma-behandeling, via een soortgelijk procédé als het hydrophiel maken van het oppervlak. (In feite is dat geen toeval, omdat een -OH groep sterk hydrophiel is.) Door nu een chlorosilaanmolekuul, met daaraan een staart bestaande uit een gefluorideerde koolstofketen op het met plasma behandelde oppervlak los te laten, zou er een soort teflon-coating op de prothese moeten ontstaan.

ESCA-metingen, waarbij de samenstelling van het oppervlak in chemische elementen wordt bepaald, wezen zeer overtuigend uit dit inderdaad het geval was. Dit werd bevestigd door de uitkomsten van zogenaamde Fourier Transform Infrared Spectroscopy, die informatie geeft over de chemische bindingen in het opervlak. Uit contacthoekmetingen bleek dat het aldus behandelde siliconenrubber nog belangrijk hydrophober was dan normaal (**hoofdstuk 7**). Uiteraard moest ook dit nieuwe materiaal weer *in vitro* en *in vivo* worden getest. Hierbij zagen we *in vitro* dat het behandelde siliconenrubber niet alleen minder snel begroeid raakte, maar ook dat het leiden van een luchtbelletje langs het behandelde oppervlak resulteerde in de verwijdering van praktisch alle daaraan gehechte micro-organismen. Dit was in het bijzonder het geval indien het siliconenrubber was voorbehandeld met speeksel, wat natuurlijk de situatie in de keelholte beter weergeeft. Het is bekend dat een dergelijk luchtbelletje een zeer grote afschuifkracht aan het oppervlak veroorzaakt. Bovengenoemde uitkomst duidt er dus op dat de op het behandelde oppervlak aanwezige micro-organismen zich niet meer goed kunnen vasthechten en nog maar losjes aan het oppervlak zitten. Al deze resultaten waren het meest geprononceerd als de perfluorochlorosilaanstaarten acht gefluorideerde koolstofatomen bevatten. Een coating met slechts één gefluorideerd koolstofatoom gaf een iets minder duidelijk beeld. Dit verschil is te begrijpen door de entropie aan het oppervlak in beschouwing te nemen. De lange ketens hebben

een hogere mobiliteit en daardoor een hogere entropie. Kennelijk geeft de combinatie van hoge entropie en hydrophobiciteit goede "anti-fouling" eigenschappen aan het siliconenrubber.

De tot nu toe gedane *in vivo* experimenten wijzen in dezelfde richting (**hoofdstuk 8**). De drie geëxplanteerde Groninger knoopjes die met korte ketens waren behandeld gaven weinig verschil te zien tussen behandelde en onbehandelde kant en waren soms zelfs sterker begroeid op de behandelde kant. Maar de knoopjes die met lange perfluorketens waren behandeld toonden zo op het oog minder aangroei op de behandelde kant. Door de aangroeisels van elke kant af te krabben en op petrischalen te kweken konden de aantallen en de soorten micro-organismen op beide kanten nauwkeurig worden vergeleken. Hierbij bleek dat er inderdaad minder micro-organismen zaten op de kant die was behandeld met lange perfluorketens.

We hebben nu dus een methode in handen om de overwoekering van de stemprothese belangrijk te verminderen, waardoor de levensduur, naar alle waarschijnlijkheid, kan worden verlengd. De in dit proefschrift beschreven split-button techniek geeft ons daarbij een ideaal instrument in handen om het effect van een behandeling snel en nauwkeurig *in vivo* te beoordelen. Dat het chemisch behandelen van het oppervlak van een stemprothese in ieder geval vanuit het oogpunt van de drager een aantrekkelijke aanpak is, werd mij duidelijk uit de reaktie van bovengenoemde patiënt toen ik hem vertelde waar ik mee bezig was: "*Hardstikke goed nieuws, ik heb altijd grote moeite met wisselen (...). Ook vind ik het vervelend om elke dag pillen te moeten slikken. Daarom vind ik uw methode ideaal, want dan zou ik de prothese gewoon kunnen vergeten.*"

RESUME

Le traitement du cancer du larynx ou de l'hypopharynx par laryngectomie affecte sévèrement l'élocution, la déglutition et la respiration. Au cours des deux dernières décennies, les procédés chirurgicaux de restauration vocale basés sur la création d'un pertuis trachéo-oesophagien avec insertion d'une prothèse vocale ont grandement amélioré les perspectives de recouvrement de la voix après laryngectomie (voir le **chapitre 1** pour un état de la littérature). La plupart des prothèses sont constituées de caoutchouc silicone en raison des excellentes propriétés mécaniques et des bonnes caractéristiques de moulage de ce matériau. L'inconvénient majeur de ces prothèses provient de la colonisation microbienne. D'un point de vue clinique, le remplacement des prothèses vocales s'impose lorsque le patient se plaint d'une perte d'étanchéité à la nourriture ou aux boissons ou encore d'une résistance accrue au passage de l'air.

L'infection des prothèses est un problème médical majeur. Comme l'adhésion microbienne au dispositif implanté dépend de ses propriétés de surface, leur modification pourrait éventuellement prévenir la formation d'un biofilm. Le caoutchouc silicone est un matériau hydrophobe, c'est pourquoi nous avons d'abord tenté d'en rendre la surface hydrophile, afin d'en accroître la résistance à la colonisation. Le traitement par plasma RF (radio fréquence) est une méthode souvent efficace d'hydrophilisation des surfaces de polymères, mais les effets d'un traitement unique sont passagers en raison de la mobilité élevée du squelette siloxane. Aux **chapitres 2 et 3** nous proposons une solution à ce propblème du retour à l'état hydrophobique.

Au **chapitre 2**, nous décrivons les résultats de l'étude des propriétés de surface de caoutchouc silicone modifié par des traitements plasma répétés à l'oxygène, à l'argon, au dioxyde de carbone et à l'ammoniac. Les échantillons traités ont été stockés à l'air avant d'être caractérisés par mesures d'angle de contact (H_2O), par spectrométrie de photoélectrons induite par rayonnement X (XPS), par mesures de potentiel zéta et par profilométrie. Les traitements par plasma d'argon, de dioxyde de carbone et d'ammoniaque diminuent fortement l'angle de contact avancant de 115 à respectivement 58, 72 et 85 degrés, cet effet est durable en cas de traitement répété. Par contre, les effets d'un traitement par plasma d'oxygène disparaissent au bout de cinq heures, même en cas de traitement répété. La formation au cours du traitement plasma de ponts Si-O-Si entre chaînes siloxanes a pu être confirmée par l'observation d'une nouvelle composante du pic Si_{2p}, avec des intensités variables, selon le gaz utilisé. Les mesures de potentiel zéta indiquent que les échantillons traités ont une surface plus négative que celle des échantillons non-traités. La rugosité R_A des échantillons traités de façon répétée croît légèrement, passant de 0.35 à 046 µm, tandis que l'observation au microscope électronique révèle la présence de petites fissures sur toute la surface après traitements répétés.

Au **chapitre 3**, nous exposons les résultats d'une étude comparative des conditions de retour à l'état hydrophobe de surfaces soumises à des traitements répétés et conservées à l'air, sous eau ou

sous azote liquide. La dépendance temporelle des effets du traitement sur les propriétés physico-chimiques du caoutchouc silicone ont été étudiées par mesures d'angle de contact et par XPS. Quel que soit le type de plasma utilisé, après trois mois le retour à l'état hydrophobique des échantillons conservés à l'air est quasi-total. Dans le cas d'un stockage sous azote liquide, le retour à l'état hydrophobique peut être considérablement atténué, ce qui se traduit par une faible modification de l'angle de contact avancant, quel que soit le type de plasma. De même, dans le cas des échantillons consevés sous eau, le retour à l'état hydrophobique est minime, mais reprend avec la remise à l'air. L'analyse XPS montre que les échantillons traités à l'argon, au dioxyde de carbone et à l'ammoniac et conservés sous eau ou sous azote liquide présentent tous une proportion accrue de carbone, aux dépens de l'oxygène et du silicium, à l'encontre de ceux laissés à l'air. Notons aussi que la teneur en carbone du caoutchouc silicone traité par plasma d'oxygène diminue lors de la conservation sous eau ou sous azote liquide et que la teneur en oxygène et en silicium décroît, par opposition à ce qui est observé pour un stockage à l'air.

Au **chapitre 4**, nous donnons les résultats de l'étude quantitative de la restructuration de la surface d'échantillons de caoutchouc silicone soumis à des traitements répétés de plasmas de divers gaz tels que l'oxygène, l'argon, le dioxyde de carbone et l'ammoniac. Les fractions superficielles couverte par des groupes polaires mobiles ou immobiles ainsi que l'échelle de temps du processus de restructuration de la surface ont été obtenues par des de mesures d'angles de contact avancant et au retrait (voir chapitre 2). Dans le cas des échantillons traités par plasma d'argon, la fraction de groupes polaires immobiles augmente avec le nombre de répétitions du traitement, mais ne varie guère dans le cas du traitement par un plasma d'ammoniac. L'utilisation d'un plasma d'oxygène conduit à l'incorporation de groupes polaires mobiles mais pas de groupes immobiles. L'allongement du temps caractéristique de restructuration du caoutchouc silicone traité par plasmas d'argon ou d'ammoniac en fonction du nombre de traitements effectués indique que ces traitements augmentent le nombre de liaisons entre chaînes. Dans le cas du traitement au plasma d'oxygène, le temps cartactéristique de restructuration diminue, ce qui correspond à une rupture des chaînes provoquée par le plasma.

Au **chapitre 5**, nous discutons les effets du caoutchouc silicone hydrophilisé sur l'adhésion *in vitro* et sur la croissance de bactéries et de levures provenant de prothèses vocales, ainsi que la formation *in vivo* du biofilm. Nous montrons que l'adhésion microbienne *in vitro* et la croissance à la surface du caoutchouc silicone peuvent être réduites par traitement plasma à l'argon. Toutefois, la formation du biofilm *in vivo* se trouve favorisée par l'hydrophilisation de la surface des prothèses. Ceci nous amène à conclure que l'hydrophobicité des biomatériaux utilisés est un paramètre important du contrôle de la formation de biofilms à la surface des prothèses vocales.

Le **chapitre 6** présente une méthode nouvelle permettant d'effectuer *in vivo* une évaluation rapide et précise du profil longitudinal de la formation du biofilm à la surface de prothèses vocales

traitées. Cette méthode est basée une modification de la prothèse de Groningue (aussi dénomée "Groningen button"), dont une moitié seulement est soumise au traitement par plasma. On obtient ainsi des prothèses dont un côté est hydrophilisé tandis que l'autre, non modifié, sert de témoin. Cette méthode est également appelée "split-button". Nous avons observé un contraste prononcé entre les deux régions avec une augmentation clairement perceptible de la colonisation de la région hydrophilisée. Cette méthode de traitement partiel s'est avérée très utile pour éliminer l'influence d'autres facteurs tels que les habitudes alimentaires des divers patients. L'analyse microbiologique des biofilms correspondant à chacune des deux régions de la prothèse n'a pas mis en évidence de différence des populations les plus communes: *Candida albicans*, streptocoques et staphylocoques.

Comme nous l'avons indiqué aux chapitres 5 et 6, la formation de biofilms *in vivo* se trouve favorisée par l'hydrophilisation de la surface du catchouc silicone, c'est pourquoi nous avons entrepris la fabrication d'autres surfaces combinant une hydrophobie et une entropie élevées. Au **chapitre 7**, nous étudions l'adhésion *in vitro* de levures et de bactéries à la surface de caoutchouc silicone rendu fortement hydrophobique par chimisorption de chaînes courtes ou longues de polymères d'alkylsiloxane fluoré. Les propriétés physico-chimiques de la couche chimisorbée ont été étudiées par mesures d'angles de contact, par photoémission (XPS) et par spectroscopie par transformée de Fourier en réflexion totale atténuée (ATR-FTIR). L'adhésion sur ces couches de *Streptococcus salivarius*, de *Staphylococcus epidermidis*, de *Candida albicans* et de *Candida tropicalis* extraits de prothèses utilisées a été étudiée *in vitro*, tant en la présence qu'en l'absence d'un film salivaire. Les surfaces traitées se caractérisent par une adhésion microbienne atténuée, aussi bien au début du processus que lorsque l'état stationnaire est atteint. De plus, les organismes adhérents sont plus aisément détachables par l'application d'une interface air-liquide. La réduction d'adhésion microbienne la plus importante a été observée dans le cas de chaînes fluorocarbonnées longues, qui combinent une énergie libre de surface peu élevée avec une entropie superficielle forte.

Au **chapitre 8**, nous présentons les résultats de l'étude *in vivo* de la formation de biofilms à la surface de prothèse vocales traitées par l'adsorption de chaînes longues d'alkylsiloxane perfluoré, selon le protocole expérimental décrit précédemment. Nous avons observé une formation réduite de biofilms à la surface des prothèses rendues hydrophobes, le traitement par chimisorption de chaînes d'alkylsiloxane perfluoré est donc susceptible d'allonger le temps de vie des prothèses vocales. L'analyse microbiologique effectuée des deux côtés de la bride oesophagienne de la prothèse montre que les populations en levure sont très semblables, *Candida* spp étant la plus fréquemment observée. Pour ce qui concerne les bactéries, le nombre d'espèces mises en évidence est sensiblement supérieur du côté non traité, avec une dominance des staphylocoques.

Au **chapitre 9**, à l'issue de la discussion générale de cette thèse, nous formulons des

suggestions quant aux moyens d'éviter la colonisation des prothèses vocales en caoutchouc par les micro-organismes.

En résumé, cette thèse démontre que l'utilisation de la méthode dite du "split-button" développée au cour de cette thèse, est plus probablement la seule méthode permetant d'évaluer d'une manière efficace et rapide la formation du biofilm ayant lieue *in vivo* à lar surface des prothèses vocales en silicone. De plus, nous avons démontré que grâce à des traitements de *surfaces* appropriés, nous pouvons atténuer la colonisation des prothèses vocales et de ce fait accroître leur durée de vie, ce qui ne peut qu'être profitable aux patients ayant subi une laryngectomie. Aussi, je laisserai le dernier mot à un patient (après lui avoir relaté le but de ma recherche): "*... c'est une très bonne nouvelle car dans mon cas, le changement régulier de la prothèse de Groningue se fait toujours de manière pénible. (...). Je trouve que votre méthode est idéale car dans ce cas, je pourrais tout simplement oublier la prothèse. (...) Le fait d'être contrain d'avaler 3 à 4 fois par jour une pillule anti-fungale m'ennuie énormément et de plus peut nuire à ma santé...*"

CURRICULUM VITAE

Emmanuel P.J.M. Everaert was born on April 29th, 1966 in Namur, Belgium. In 1985, he accomplished his high school degree at the Collège Notre Dame-de-la paix, Erpent, Namur, Belgium. Specialising in Surface Sciences and in Physico-Chemistry, he received his Master Degree in Chemical Sciences from the University of Namur, Belgium, in June 1990.

In August 1991, he finished a second Master Degree the field of Polymer Materials at the University of Louvain-la-Neuve, Belgium, where he wrote a thesis in the Surface Sciences field with specials applications in the biocompatibility of polymer membranes.

From 1993 until 1997 he worked on his Ph-D at the University of Groningen, Laboratory of Materia Technica, Groningen, The Netherlands.